



UNIVERSITY OF KWAZULU-NATAL

**Severe Acute Malnutrition and Antiretroviral Treatment in
Children with HIV**

Submitted By

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In fulfilment of the requirements for the degree of Doctor of Philosophy

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PREFACE

While treating children with HIV in KwaZulu–Natal, South Africa, I noted the high mortality and the difficulties in managing severely malnourished HIV-infected children. On review of the literature there was a lack of evidence supporting management guidelines. This thesis emanates from a desire to provide evidence to guide clinical practice and change management guidelines of severely malnourished HIV-infected children.

DECLARATION: PLAGIARISM

I, Moherndran Archary declare that:

- i. The research reported in this dissertation, except where otherwise indicated, is my original work.
- ii. This dissertation has not been submitted for any degree or examination at any other university.
- iii. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
- iv. This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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DECLARATION: PUBLICATIONS

I, Dr Moherndran Archary, declare as follows:

i. That the work described in this thesis has not been submitted to UKZN or other tertiary institution for purposes of obtaining an academic qualification, whether by myself or any other party.

ii. That my contribution to the project was as follows:

a) Archary M, Adler H, La Russa P, Mahabeer P, Bobat R. Bacterial infections in HIV-infected children admitted with severe acute malnutrition in Durban, South Africa. *Paediatr Int Child Health*. 2016;Jul 4:1-8.

I designed the protocol and data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, cleaned and analysed the data, and drafted and revised the paper.

b) Archary M, Sartorius B, La Russa P, Sibaya T, Healy M, Bobat R.A. HIV-infected children with severe acute malnutrition: a randomized controlled clinical trial comparing early versus delayed initiation of antiretroviral treatment. Submitted to: *International Journal of Antimicrobial Agents*, IJAA-D-16-01055

I designed the protocol and data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, cleaned and analysed the data, and drafted and revised the paper

c) Moherndran Archary, Helen McIlhleron, Raziya Bobat, Phillip La Russa, Thobekile Sibaya, Lubbe Wiesner, Stefanie Hennig. Population pharmacokinetics of lopinavir in severely malnourished HIV-infected children and the effect on treatment outcomes. Submitted to: *the Paediatric Infectious Diseases Journal*, PIDJ-216-994.

I designed the protocol and data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, cleaned and analysed the data, and drafted and revised the paper),

- d) Adler H, Archary M, Mahabeer P, LaRussa P, Bobat RA. Tuberculosis in HIV-infected South African children with complicated severe acute malnutrition. Submitted to: International Journal of Tuberculosis and Lung Disease. Submitted to IJTLD, 10-16-0753.

I designed the protocol and data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, assisted with drafted and revised the paper

- e) Muenchhoff M, Healy M, Singh R, Roidera J, Groll A, Kindra C, Sibaya T, Moonsamy A, McGregor C, Phan MQ, Palma A, Klooverpris H, Leslie A, Bobat R, LaRussa P, Ndung'u T, Goulder P, Sobieszczyk ME, Archary M. Malnutrition increases microbial translocation, systemic immune activation and immune exhaustion and impairs immune recovery in HIV-infected children. Submitted to: AIDS Research and Human Retroviruses, AID-2016-0261.

I designed the protocol and data collection tools, monitored data collection for part of the trial, assisted with cleaning and analysing the data, and assisted with drafting and revising the paper

Signed _____

Date _____

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DEDICATION

Dedicated to my sons, Thashir and Shayur and my parents for their strength and
inspiration.

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ACRONYMS

ABC	Abacavir
ALT	Alanine Transaminase
ART	Antiretroviral treatment
ESBL	Extended Spectrum Beta-Lactamase
CFU	Colony Forming Units
Cl	Clearance
Cl/F	Apparent Clearance
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
DNA PCR	Deoxyribonucleic Acid Polymerase Chain Reaction
EFV	Efavirenz
EID	Early Infant Diagnosis
ELISA	Enzyme Linked Immunosorbent Assay
FFM	Fat-Free Mass
HAI	Hospital Acquired Infection
HAZ	Height for Age Z score
HIV	Human Immunodeficiency Virus
IRIS	Immune Reconstitution Inflammatory Syndrome
KEH	King Edward VIII Hospital
LAMIC	Low and Middle Income Countries
LPA	Line Probe Assay
LPV	Lopinavir
LPV/rtv	Lopinavir/ritonavir
MAM	Moderate Acute Malnutrition
MTB	Mycobacterium Tuberculosis
MTB DRplus	Mycobacterium Tuberculosis Drug Resistance plus
MUAC	Mid Upper Arm Circumference
NVP	Nevirapine
Pk	Pharmacokinetic
PMTCT	Prevention of Mother To Child Transmission
REE	Resting Energy Expenditure
SAHANES	South African National Health and Nutrition Examination Survey
SAM	Severe Acute Malnutrition
UNAIDS	The Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations Children's Emergency Fund

VL	Viral Load
WHO	World Health Organisation
WHZ	Weight for Height Z score
WAZ	Weight for age Z score

ABSTRACT

Background:

Childhood malnutrition remains a common problem in many parts of the world and is a contributing factor in 45% of the 5.9 million annual deaths in children under 5 years. HIV-infected children have a disproportionately higher prevalence of malnutrition and higher mortality associated with malnutrition as compared to non-infected children. Physiological changes associated with malnutrition and re-nutrition complicate antiretroviral treatment in these children. This thesis explores aspects related to antiretroviral treatment (ART) in severely malnourished HIV-infected children, including the timing of ART initiation, pharmacokinetics of antiretroviral drugs, co-infections with bacterial and mycobacterial infections and the effect of microbial translocation on immune restoration.

Methods:

Eight-two patients were enrolled in this randomized controlled trial, where HIV-infected children admitted with severe acute malnutrition (SAM) were initiated on ART either early (within 14 days of admission) or delayed (after 14 days with evidence of nutritional recovery). Clinical and laboratory parameters were collected during the admission and patients were followed up at 4, 8, 12, 24 and 48 weeks post admission. A pharmacokinetic evaluation of lopinavir (LPV) was conducted on Day 1 and 14 of ART initiation. Samples for evaluation of microbial translocation and immune restoration were collected in 32 study patients and 75 additional patients in 3 control groups.

Results/Discussion:

There were no significant differences in immunologic, virologic or anthropometric responses at 48 weeks between the early and delayed arms. However, significantly improved rates in the changes in viral load, WAZ (weight-for-age Z score) and HAZ (height-for-age Z score) favoured the delayed arm.

Pharmacokinetic (pk) evaluation of the LPV, displayed significant pk variability, reduced bioavailability and consequently greater apparent clearance (CL/F) estimates in comparison to other pk studies of LPV in non-malnourished children. Fat-free Mass (FFM) was shown to affect LPV variability; however delay in ART initiation and “super-boosted” LPV/rtv did not affect LPV variability.

Bacterial pathogens were identified in 51% of patients. Of the hospital acquired infections (HAI), 41% were extended spectrum beta-lactamase (ESBL)-producing gram-negative infections. Tuberculosis (TB) co-infection was common (25.6%), with bacteriological confirmation in 38% of treated cases.

Malnutrition was associated with increased microbial translocation, immune activation and immune exhaustion, with a negative impact on immune recovery in HIV-infected children on ART.

Conclusions:

Delaying ART initiation to at least 14 days after starting nutritional support is associated with improved rates of clinical (changes in WAZ and HAZ) and virologic outcomes. However this delay did not improve LPV exposures and dose adjustment of LPV during nutritional recovery needs to be further evaluated. These results can be used to inform changes in clinical practice and national and international guidelines for the management of severely malnourished HIV-infected children.

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LIST OF PUBLICATIONS EMANATING FROM THIS DISSERTATION

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Moherndran Archary (designed the protocol and data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, cleaned and analysed the data, and drafted and revised the paper), Helen McIlherson (assisted with design of the protocol and revision of the paper), Raziya Bobat (assisted with design of the protocol and revision of the paper), Phillip La Russa (assisted with design of the protocol and revision of the paper), Thobekile Sibaya (monitored data collection for the whole trial), Lubbe Wiesner (monitored sample collection for the whole trial), Stefanie Hennig (assisted with cleaning and modeling of the data, and assisted with the drafting and revision of the paper).

- iv. Adler H, Archary M, Mahabeer P, LaRussa P, Bobat RA. Tuberculosis in HIV-infected South African children with complicated severe acute malnutrition.**

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Adler H (wrote the statistical analysis plan, cleaned and analysed the data, and drafted and revised the paper), Archary M (designed the protocol and data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, assisted with drafting and revision of the paper), Mahabeer P (cleaned the data and revision of the paper), La Russa P (assisted with design of the protocol and revision of the paper), Bobat R (assisted with design of the protocol and revision of the paper)

- v. **Muenchhoff M, Healy M, Singh R, Roidera J, Groll A, Kindra C, Sibaya T, Moonsamy A, McGregor C, Phan MQ, Palma A, Kloverpris H, Leslie A, Bobat R, LaRussa P, Ndung'u T, Goulder P, Sobieszczyk ME, Archary M. Malnutrition increases microbial translocation, systemic immune activation and immune exhaustion and impairs immune recovery in HIV-infected children. Submitted to: AIDS Research and Human Retroviruses #AID-2016-0261.**

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LIST OF CONFERENCE PRESENTATIONS

1. Adler H, Archary M, Mahabeer P, La Russa P, Bobat R and the MATCH study team. Two's company, three's a crowd: TB in HIV-infected South African children with complicated severe acute malnutrition. 47th Union World Conference: Abstract 272, 28 October 2016.
2. Archary M, Hennig S, Wiesner L, La Russa P, Bobat R, McIlleron H and MATCH study team. Population Pharmacokinetics of Lopinavir/ritonavir in Severely Malnourished HIV-infected children: Early versus delayed initiation of antiretroviral treatment. Presented at 8th HIV Pediatric Workshop, Durban, South Africa, 15 and 16 July 2016
3. Archary M, Adler H, Bobat R, Larussa P and MATCH Study team. WSPID - 0845. Bacterial Infections in HIV-infected children admitted with Severe Acute Malnutrition (SAM) in Durban, South Africa. Rio de Janeiro, Brazil, from 18-21 November, 2015.
4. Archary M, Bobat R, La Russa P and MATCH Study team. Severe acute malnutrition and timing of anti-retroviral initiation in children with HIV. A 24-week analysis comparing early vs. delayed initiation of ART in severely malnourished HIV-infected children. Presented at AIDS 2014 – Melbourne, Australia, July 2014
5. Archary M, Bobat R, La Russa P and MATCH Study team. Severe acute malnutrition and timing of anti-retroviral initiation in children with HIV. A 24-week analysis comparing early vs. delayed initiation of ART in severely malnourished HIV-infected children. Presented at 6th HIV Pediatric Workshop, 18 and 19 July 2014 – Melbourne, Australia.

CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 *Epidemiology of Paediatric HIV infection and treatment*

UNAIDS estimated that, in 2015, there were 36.7 million people living with HIV globally and 2.1 million new infections (1). This represented a six percent reduction in new adult infections and a 50% reduction in new paediatric infections compared to 2010 (2). This large reduction in new paediatric infections is due in part to the expanded access to antiretroviral treatment (ART) in women for Prevention of Mother to Child Transmission (PMTCT). However the drop fell short of the 90% target set by the World Health Organisation (WHO) in 2010 (3) and resulted in an estimated 150 000 newly infected children globally, more than a third of whom resided in eastern and southern Africa (4).

Disparate access to ART across regions is vast, with over 95% of children in western and central Europe and North America accessing ART in contrast to 20% in northern and central Africa and 63% in southern and eastern Africa (1). Lack of or limited access to, early infant diagnosis (EID) and paediatric ART services contribute to these differences and to more advanced HIV disease at ART initiation (5). As a result, malnutrition is a common presenting clinical problem in HIV-infected children in low and middle income countries (LAMIC) and is often the initiating event prompting health-care seeking behavior (3,6,7).

1.1.2 *Epidemiology of malnutrition in children*

Malnutrition can be evaluated using invasive and non-invasive methods including measurement of lean body mass/fat free mass, albumin and other serum markers. For this dissertation, anthropometry (Z-scores) was chosen, as it is the most widely used method of evaluating malnutrition in clinical practice and in the literature. UNICEF estimates that globally there are approximately 48.8 million children under the age of 5 years who have wasting (Weight for Height Z-score <2). While less than 1% of children in North America and Australia are wasted, in Southern Asia and Africa the percentage is over 5% (8).



Source: UNICEF, WHO, World Bank Group joint malnutrition estimates, 2016 edition.
 Note: * Eastern Asia excluding Japan; ** Oceania excluding Australia and New Zealand, *** Australia and New Zealand, regional average based on Australian data, **** Northern America regional average based on United States data. These maps are stylized and not to scale and do not reflect a position by UNICEF, WHO or World Bank Group on the legal status of any country or territory or the delimitation of any frontiers.

Figure 1. Global Burden of childhood wasting (8)

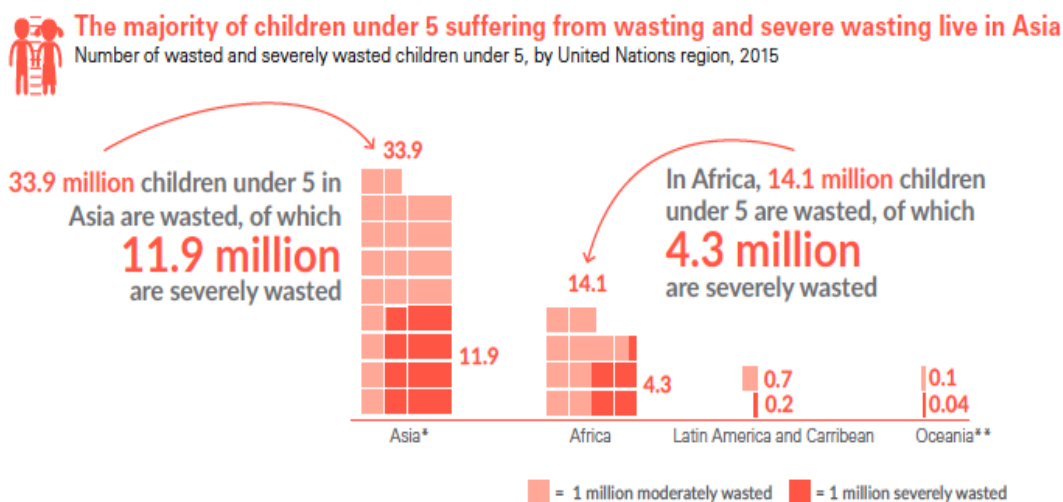


Figure 2. Regional Contributions to the burden of severe wasting (8)

Global figures for severe wasting (<-3 WHZ) are similar, with Asia and Africa contributing to the majority of the global burden (8). The South African National Health and Nutrition Examination Survey (SAHANES-1) conducted in 2012 found that 2.9% of the sampled children were wasted with 0.8% being severely wasted. In KwaZulu Natal, 2.4% were wasted and 0.1% were severely wasted (9). While anthropometry remains the standard for comparing malnutrition

1.1.3 Dual epidemic of Malnutrition and HIV

For children living in Africa, HIV and malnutrition are equally prevalent, resulting in a perfect storm. In a systematic review conducted in 2008, the overall prevalence of HIV in Sub-Saharan African children with severe acute malnutrition (SAM) was 29.2% (6). (Table 1) **The WHO definition of SAM in children 6-59 months of age is a mid-upper arm circumference (MUAC) <115 mm or weight for height < - 3 Z score of the WHO growth standard or bilateral**

oedema of nutritional origin. In children over 60 months additional criteria include body mass index < -3 Z score below the growth standard or MUAC 5-9 years < 13.5cm or < 16cm between 10-14 years.

Table 1. Studies identified in review by Fergusson et al. of HIV prevalence in children with SAM (6)

Treatment mode	Study	Reference	Research setting	Research method	HIV Prevalence (%)
Facility	A	Mgone et al. (1991)	Tanzania; paediatric ward, large referral hospital	Case-control	26/102 (25.5)
	B	Kurawige et al. (1993)	Rwanda; paediatric ward, large referral hospital	Prospective cohort	11/66 (16.7)
	C	Prazuck et al. (1993)	Burkina Faso; NRU, referral hospital	Prospective cohort	60/428 (14.0)
	D	Akenami et al. (1997)	Nigeria; hospitals and rural survey	Point prevalence	4/206 (1.9)
	E	Ticklay et al. (1997)	Zimbabwe; paediatric ward, large referral hospital	Prospective cohort	68/140 (48.6)
	F	Chintu et al. (1998)	Zambia; NRU in large referral hospital	Case-control	39/167 (23.4)
	G	Kessler et al. (2000)	Malawi; NRU in large referral hospital	Prospective cohort	86/250 (34.4)
	H	Yeung et al. (2000)	South Africa; paediatric ward, rural South African hospital	Prospective cohort	37/73 (50.7)
	I	Amadi et al. (2001, 2005)	Zambia; NRU in large referral hospital	RCT	106/196 (54.1)
	J	Bachou et al. (2006a, 2006b)	Uganda; NRU in large referral hospital	Prospective cohort	64/213 (30.0)
	K	Thurstans et al. (2008)	Malawi; 12 NRUs across Malawi, urban/rural	Point prevalence	113/522 (21.6)
	L	Chinkhumba et al. (2008)	Malawi; one urban and two rural hospital-based NRUs	Prospective cohort	79/454 (17.4)
	CTC	M	Sandige et al. (2004)	Malawi; large referral hospital-based CTC	RCT
N		Ndekha et al. (2005)	Malawi; large referral hospital-based CTC	RCT	93/375 (24.8)
O		Bahwere et al. (2007)	Malawi; CTC, rural Malawi	Prospective cohort	22/490 (4.5)
P		Kerac and Bunn (2007)	Malawi; large referral hospital (early discharge to CTC)	Chart review	355/690 (51.4)
Q		Sadler et al. (2008)	Malawi; large referral hospital-based CTC	Prospective cohort	186/259 (71.8)

CTC: community-based therapeutic care; NRU: nutritional rehabilitation unit; RCT: randomised controlled trial

In more recent studies, a similarly high incidence of malnutrition was seen in children accessing HIV care, despite improvements in HIV prevention and treatment. In a study of 1350 children from Central and West Africa accessing HIV care, 42% were malnourished with 9% being acutely malnourished (10). The TREAT Asia cohort reported that although the prevalence of SAM at ART initiation decreased from 13.5% between 2003-2006 to 8.2% between 2011-2014, this was still higher than the expected prevalence in the general population (11).

1.1.4 Pathogenesis of malnutrition in HIV-infected children

The pathogenesis of malnutrition in HIV-infected children is multi-factorial. Factors include dysregulation of Resting Energy Expenditure (REE) (12), HIV-associated wasting syndrome, chronic/persistent diarrhea, HIV-associated enteropathy, the presence of co-infections and food insecurity (13). Figure 3 provides a biological and social framework that

explores the contributing factors resulting in malnutrition in HIV-infected children. Prevention and treatment therefore requires interventions at multiple levels both biomedical and social.

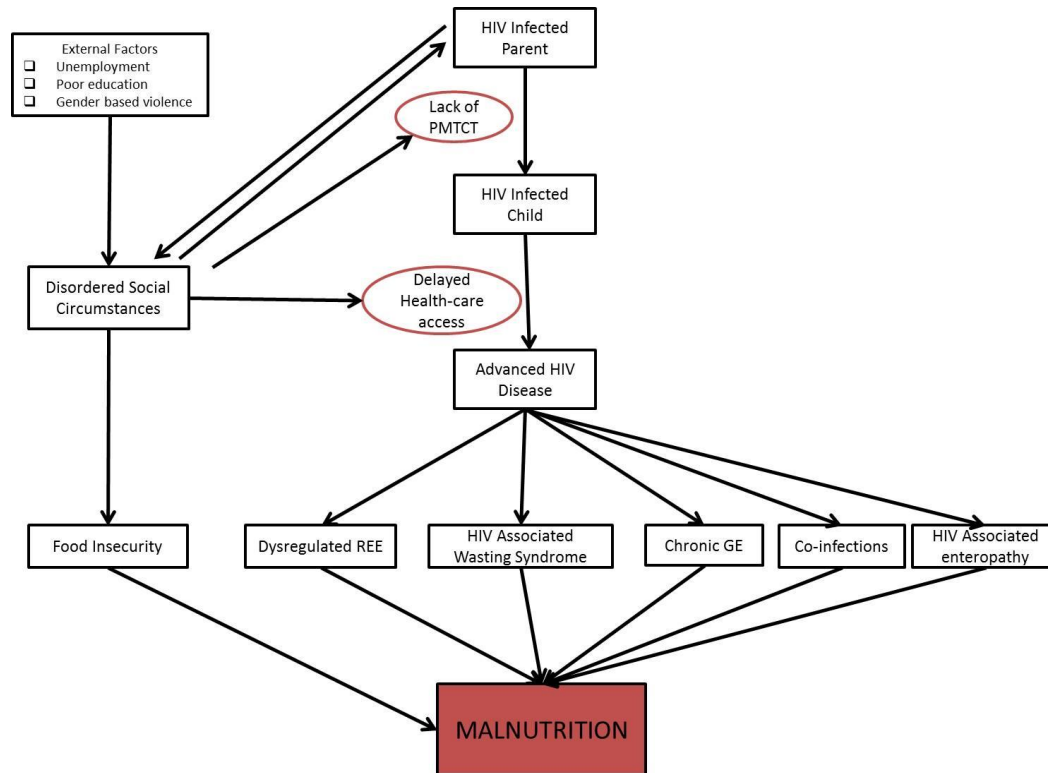


Figure 3. Biological and social framework contributing to malnutrition in HIV-infected children (designed by Dr M Archary)

Evidence based guidelines developed by the WHO for the management of severely malnourished children address many of the underlying pathogenic mechanisms including a standardised feeding schedule, routine administration of broad-spectrum antibiotics and anti-helminthics and vitamin/mineral supplementation. However, these guidelines are based on studies in HIV negative children. There is a lack of specific evidence to guide the management of HIV-infected children (14).

1.2 Literature review

1.2.1 Outcomes associated with ART initiation in malnourished children

A review of the literature for published studies evaluating and comparing mortality (Table 2) and anthropometric responses (Table 3) in HIV-infected and uninfected children was conducted.

Table 2. Comparison of mortality associated with SAM in HIV-infected and uninfected children.

No	Study	Study Setting	Sample size	Follow-up period	Mortality	
					HIV-infected	HIV Uninfected
1.	Boettiger, 2016 (11)	TREAT Asia	355	36 months	15.8%	Not reported
2.	Munthali, 2015 (15)	Zambia	9540	Inpatient mortality (2009-2013)	Overall mortality 46% with HIV-infected children 80% more likely to die (HR= 1.8, 95% CI: 1.6-1.2)	
3.	Gebremichael, 2015 (16)	Southern Ethiopia	929	Inpatient mortality (mean admission 26 days)	9.3% (did not differentiate bet HIV positive and negative children)	
	Asafo-Agyei, 2013 (17)	Ghana	247 (67)	Inpatient mortality	37.8%	10.1%
4.	Madec, 2011 (10)	Niger	477	Inpatient mortality	20%	14%
5.	Fergusson (systematic review), 2009 (6)	Sub-Saharan Africa	3327	Variable	30.4%	8.4%

Table 3. Comparison of growth responses of HIV-infected and uninfected children with SAM

No	Study	Study Setting	Sample size	Parameter	HIV-infected	HIV uninfected
1.	Boettiger, 2016 (11)	TREAT Asia	355	WAZ	-5.6 (baseline) to -2.8 (48 weeks)	Not reported
				CD%	3% (baseline) to 12% (48 weeks)	12%
2	Asafo-Agyei, 2013 (17)	Ghana	247 (67)	Mean weight gain/kg/day	2.4g/kg/day	7 g/kg/day
3	Madec, 2011 (10)	Niger	477	Duration of re-nutrition	22 days	15 days

Survival and growth outcomes of malnourished HIV-infected children are worse when compared to their non-infected counterparts. Nutritional rehabilitation is insufficient on its own to improve outcomes, and, even with ART initiation, malnutrition has been associated with higher mortality (18) and delayed immunological recovery (19). In a retrospective chart review of patients initiated on ART in KwaZulu-Natal, wasting at initiation was found not to influence growth, immune and virologic responses (20), however the effect of mortality and loss to follow-up was not assessed.

The reasons for the differences in outcomes between HIV-infected and uninfected severely malnourished children are not known, but may be related to altered pharmacokinetics in malnourished children, persistent co-infections, immunologic deficiencies associated with malnutrition (21), specific effects of micronutrient deficiency (22,23), and altered mitochondrial function (12).

1.2.1 Outcomes associated with timing of ART initiation in malnourished children

A review of the literature identified two published studies and one unpublished citation (personal communication) addressing the issue of timing of ART initiation in malnourished children (Table 4).

Table 4. Studies reporting on the effect of ART timing on outcomes in malnourished children

No	Study	Study Setting	Study Design	Sample Size	Outcome
1	Kim, 2012 (24)	Malawi – uncomplicated malnutrition/ outpatient management	Retrospective observational study	140	ART < 21 days: higher rate of nutritional recovery (86% vs 60%) and higher rate of weight gain (3.6 vs 1.6 g/kg/day)
2	Unpublished (25)	Uganda	Retrospective cohort study	345 (21% MAM or SAM)	ART < 10 weeks: higher mortality (OR:2.8, 95% CI 10.33-5.9,p=0.007)
3	Njuguna, 2016 (26)	Kenya	Randomised controlled trial	177 (32% MAM or SAM)	ART < 48hr: No difference in mortality at 24 week compared to ART at 7-14 days

The optimal timing of ART initiation in malnourished children remains unresolved. The concerns associated with ART initiation prior to nutritional improvement, are the potential for altered pharmacokinetics of antiretroviral drugs and exposure to sub-therapeutic or supra-therapeutic drug levels (27). In addition, the risk of developing Immune Reconstitution Inflammatory Syndrome (IRIS) reactions with early ART initiation is of concern (28). On the other hand, prolonged delays in ART initiation may result in excess mortality and morbidity (29).

1.2.2 Potential mechanisms for worse outcomes

1.2.2.1 Antiretroviral Pharmacokinetics in malnourished children

Appropriate drug doses are generally based on pharmacokinetic (pk) evaluations performed in well-nourished individuals. Physiological alterations in the malnourished child, including alternations in absorption of oral medications, changes in serum albumin concentrations for drug binding, and hepatic, renal and mitochondrial dysfunction can affect drug pharmacokinetics (30). In one published study, malnourished children had higher

Nevirapine (NVP) bioavailability but lower Efavirenz (EFV) and Lopinavir (LPV) bioavailability compared to **children without significant malnutrition** (31). While several studies have evaluated LPV pk's in non-malnourished children, the effect of rifampicin co-administration and the timing of ART initiation have not been evaluated.

1.2.2.2 Bacterial infections

Bacterial infections are often the precipitating events in tipping the balance toward SAM in nutritionally vulnerable children (32). Common precipitating infections include septicaemia, urinary tract infections, gastrointestinal infections and pneumonias (33). Bacterial infections are responsible for significant mortality. In a randomized controlled trial, the routine use of antibiotics in children with SAM was associated with improved recovery and decreased mortality (34).

In-patient management of HIV-infected children with SAM is complicated by the risk of acquiring nosocomial infections due to immune dysfunctions associated with malnutrition (35). Due to the lack of routine microbiological services in settings where malnutrition and HIV are common, few studies have reported on nosocomial infections in this patient population (3,33,35).

1.2.2.3 Mycobacterium Tuberculosis (MTB) Co-infection

In regions with high prevalence of HIV and malnutrition, MTB is also equally prevalent. As with other bacterial infections, MTB contributes to the pathogenesis of SAM by increasing energy requirements in nutritionally vulnerable children. In addition both HIV and malnutrition have been shown to independently increase the risk of MTB disease, however the cumulative risk has not been quantified (36,37).

Management of malnourished HIV-infected children with MTB, is complicated by cytochrome P450 enzyme induction by rifampicin and the pharmacokinetic effect on the bioavailability of antiretroviral drugs including LPV/rtv and EFV **resulting in sub-therapeutic drug concentrations in co-treated patients** (38).

1.2.2.4 Intestinal Microbiota, Bacterial Translocation and Immune activation

Malnutrition can cause an altered intestinal microbiome or be a consequence of altered microbiota (39). During malnutrition structural and functional alterations occur in the intestinal mucosa, resulting in increased permeability and translocation of bacterial products into the systemic circulation, eliciting an immune response and activation (40). This state of persistent immune activation can result in cardiovascular events and other chronic illnesses later on in life (41). The effect of ART treatment and nutritional rehabilitation in malnourished HIV-infected children on this axis has not been previously described.

1.2.2.5 Immune Reconstitution Inflammatory Syndrome (IRIS)

IRIS refers to a clinical entity characterised by a paradoxical clinical worsening of a pre-existing infectious disease following ART initiation (42). Common forms of IRIS reactions described in children include TB IRIS, BCG IRIS and skin reactions (43–46). Patients with advanced HIV disease and low CD4 counts are at higher risk of IRIS following ART initiation (47). Children with SAM are therefore at a higher risk of IRIS reactions, however few studies have reported on the frequency of IRIS in this cohort. There has been case reports of onset of oedematous malnutrition following ART initiation that was considered to be an IRIS phenomenon (48).

1.2.2.6 Refeeding Syndrome (RFS)

Children with SAM are at risk of RFS following initiation of nutritional rehabilitation (49). RFS is characterised by alterations in biochemical (hypophosphatemia, hypomagnemia and hypokalaemia) and metabolic (abnormalities in glucose metabolism and fluid balance) function (50,51). The pathophysiology of RFS is secondary to changes in insulin secretion and the resultant effects on biochemical and metabolic pathways pre and post nutritional rehabilitation. Careful control of fluid and caloric intake during the initial phase of nutritional rehabilitation, together with early recognition and treatment of RFS can improve outcomes in children with SAM (49,51).

1.3 Research problem and significance

HIV-infected children from low and middle-income countries often present with malnutrition at initial presentation with a prevalence of 42% in some cohorts (10). Several studies have demonstrated that despite ART initiation and nutritional rehabilitation, mortality rates, growth, virologic and immunologic responses are adversely affected by malnutrition when compared to non-malnourished children (6,10,11,14–16)

As highlighted in the literature review although the reasons for these differences in outcomes are not known, there are several postulated mechanisms. Malnutrition may be a surrogate marker of underlying factors such as poverty, food insecurity, poor parental education, maternal death and changing caregivers, which have been shown to independently affect outcomes (52–55). In addition several biological mechanisms have been discussed in the literature review. These include altered pharmacokinetics of antiretroviral drugs, bacterial infections, TB co-infections and alteration of the gut microbiota. The effect of delaying ART initiation until after nutritional improvement potentially will allow some of these factors to normalize and improve outcomes and reduce adverse events. Several review articles in the field have highlighted that the optimal timing of initiation of ART and the appropriate nutritional rehabilitation are priorities in the research agenda for developing countries (13,24,32,56).

The WHO nutrition advisory committee has identified the optimal timing of ART initiation, regimens and dosages in malnourished children, as areas for further research (56). Given the gaps in knowledge, the current thesis seeks to address some of these issues and assist with guiding the appropriate management of HIV-infected malnourished children.

1.4 Research Questions/Hypothesis/Objectives

1.4.1 Research Question

What effect will initiating ART after 14 days of nutritional rehabilitation with evidence of nutritional improvement have on immunologic recovery, virologic and nutritional response in comparison to earlier ART initiation, in HIV-infected children (1 month – 12 years of age) admitted with SAM.

1.4.2 Null Hypothesis

Timing of ART initiation (early versus delayed) in newly diagnosed HIV-infected children presenting with SAM will have no effect on immunologic recovery, virologic and nutritional response.

1.4.3 Objectives

1.4.3.1 Primary Objectives

- a) To compare the immunologic and virologic responses* to ART at 48 weeks in HIV-infected severely malnourished children started immediately on ART versus children in whom ART is delayed.
- b) To compare the nutritional response* at 48 weeks in HIV-infected severely malnourished children started immediately on ART versus children in whom ART is delayed.

1.4.3.2 Secondary Objectives

- a) To compare the mortality at 48 weeks in HIV-infected severely malnourished children started immediately on ART versus children in whom ART is delayed.
- b) To describe the incidence and spectrum of adverse events related to the initiation of ART in severely malnourished children.
- c) To determine the pharmacokinetics of Lopinavir in severely malnourished children **on Lopinavir/ritonavir**.
- d) To describe the spectrum of bacterial pathogens isolated in HIV-infected severely malnourished children within the first 30 days of admission.
- e) To describe the frequency and spectrum of tuberculosis co-infection in HIV-infected severely malnourished children.
- f) To evaluate the effects of HIV-infection, severe acute malnutrition (SAM) and active TB disease on microbial translocation and consequentially on immune activation and immune exhaustion in HIV-infected children.

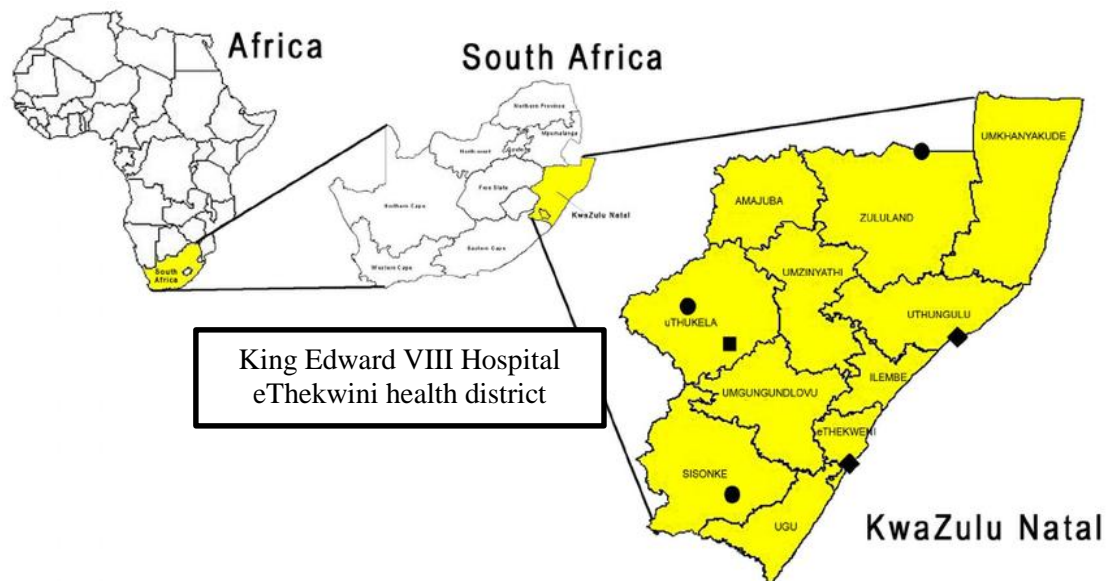
**Definitions: Virologic treatment response was defined as a HIV viral load <1000 copies/ml at 48 weeks. Immunologic treatment response was defined as an improved CD4 count to WHO Immune stage one and two. Anthropometric treatment response was defined as body mass index Z-score (BMIZ) or WHZ score of greater than -1 below the mean.*

1.5 Methodology

1.5.1 Study Design

The primary study was a prospective, randomized, controlled clinical trial.

1.5.2. Study site



Available from: https://www.researchgate.net/figure/236207880_fig1_Figure-1-Geographical-location-of-KwaZulu-Natal-with-the-six-study-areas-indicated [accessed 05 November 2016]

Figure 4. Geographical location of King Edward VIII Hospital, Durban, KwaZulu-Natal

King Edward VIII hospital (KEH) is a regional/tertiary level hospital with 100 paediatric beds across 4 wards including a nutritional rehabilitation ward. The hospital is a referral hospital, based in the most populous district in KZN with a population of approximately 3.5 million (57).

1.5.3 General Subject management

1.5.3.1 Recruitment/Inpatient Management

Patients admitted to KEH, who met the inclusion and exclusion criteria (Table 5) were eligible to be enrolled into the study. Children between the age of 1 month to 12 years were eligible for inclusion. The study age criteria was designed to reflect the real world scenario, where very young HIV-infected children presenting with symptomatic HIV disease in the first year of life (rapid progressors) and older children with a missed HIV diagnosis (intermediate or slow progressors) present to health care facilities with advanced HIV disease and SAM.

The anthropometric measurements of premature infants were plotted at their chronological age, corrected for gestational age during the first year of life. Thereafter premature children were plotted at their chronological age.

Table 5. Inclusion/Exclusion Criteria

Inclusion criteria	Exclusion Criteria
Age between 1 month to 12 years	Enrolled in other interventional studies
HIV infection defined as a positive ELISA or Rapid HIV test in children over 18 months or a positive DNA PCR in children under 18 months	
ART-naïve except for antiretroviral prophylaxis given for PMTCT	Lack of availability of parent/guardian willing and able to provide informed consent and adhere to study protocol
Severe acute malnutrition (SAM) by WHO criteria: Weight-for-length z-scores -3 below the WHO growth standards median, or mid-upper arm circumference (MUAC) < 115 mm, or peripheral oedema.	
Eligible for initiation of ART by the SA national treatment guidelines.	

The parent/legal guardian was approached and informed about the study. Informed consent was signed prior to performing any study related procedures. Routine care was provided by clinical staff. A standardized management protocol for the inpatient management of severely malnourished children was used (Appendix 1). Patients were fed a lactose-free ready-to-use formula (Appendix 2), which provided an equivalent nutritional content due to the lack of availability of the WHO specified feeds (F-75/F-100) with the following energy and protein content (Table 6).

Table 6. Energy and Protein content of feeds used during nutritional rehabilitation

Day post admission	Energy (Kcal/kg)	Protein (g/kg)
Day 1-2	33 - 37	1
Day 3-4	50-56	1.5
Day 5-6	67-74	2
Day 6-7	83-93	2.5
> Day 7	100-112	3

Following study enrolment, subjects were randomised to one of two arms (Early vs Delayed arms). Criteria for early and delayed arms are shown in Table 7.

Table 7. Criteria for early and delayed arm

Early arm	Delayed arm
ART initiated within 14 days of admission to the hospital for management of severe acute malnutrition.	ART initiated after more than 14 days of admission to the hospital for management of severe acute malnutrition and Weight/length (for infants < 87 cm) or weight/height (for infants >87 cm of -2 z-score or Achieving at least 15% weight gain or Resolution of oedema + return of appetite

Randomisation was communicated to hospital clinical staff responsible for routine clinical care of the patients. All inpatient management was at the discretion of the treating clinician.

1.5.3.2 Antiretroviral regimen

< 3 years or < 10kgs	ABC + 3TC + LPV/rtv
> 3 years and > 10kgs	ABC + 3TC + EFV

Drug doses were prescribed as per the South African National ART guidelines (2012-2015) based on the WHO weight band dosage charts. Patients on rifampicin and LPV/rtv were prescribed additional ritonavir (“super-boosted” LPV/rtv) as per the weight banded dosage table (Appendix 3). The criteria for ART initiation was as per the South African National ART guideline (2012-2015).

Following discharge from hospital, subjects were reviewed at weeks 4, 8, 12, 24 and week 48.

1.5.4 Management related to specific objectives

Specific management related to the specific objectives will be discussed in the individual chapters.

1.5.5 Ethical issues

The study was approved by the Postgraduate Committee of the University of KwaZulu-Natal (Appendix 4), the Biomedical Research and Ethics Committee (BREC) of the University of KwaZulu-Natal – BFC 126/11 (Appendix 5) and the Department of Health – King Edward VIII Hospital (Appendix 6) and KwaZulu-Natal Department of Health (Appendix 7).

Parents or legal guardians of all study participants signed an informed consent (Appendix 8) prior to any study related procedures being performed. Informed consent was conducted in English or isiZulu depending on the care-givers’ preference (Translation Certificate – Appendix 9).

1.6 Overview of the Thesis

The thesis is presented in the form of 5 articles (Chapter 2 – 6) and a synthesis chapter (Chapter 7)

Chapter 2: *HIV-infected children with severe acute malnutrition: a randomized controlled clinical trial comparing early versus delayed initiation of antiretroviral treatment.*

In this chapter a comparison of the treatment outcomes of severely malnourished children randomised to either early or delayed ART initiation is presented. The differences in mortality, adverse events, growth responses (WAZ, HAZ and WHZ), immune recovery and virologic responses between the two arms are presented. A random effects logistic regression is presented to compare the responses by trial arms. The manuscript was submitted for publication to International Journal of Antimicrobial Agents, IJAA-D-16-01055 and is under review

Chapter 3: *Population pharmacokinetics of lopinavir in severely malnourished HIV-infected children and the effect on treatment outcomes*

A pharmacokinetic model is presented that describes the lopinavir pharmacokinetics in severely malnourished children. The effect of baseline factors (age, sex), trial arm, co-treatment with rifampicin and anthropometry on lopinavir pharmacokinetics is described. Further, the effect of lopinavir pharmacokinetics on treatment failure (Death or Viral load >1000 c/ml) or treatment success (alive and Viral load < 1000c/ml) at 12 and 48 weeks is explored. The manuscript was submitted to the Paediatric Infectious Diseases Journal, PIDJ-216-994 and is under review

Chapter 4: *Bacterial infections in HIV-infected children admitted with severe acute malnutrition in Durban, South Africa*

In this chapter the frequency and bacteriological characteristics (identification and antibiotic susceptibility) of pathogens isolated during the first 30 days following admission is described. The impact of bacterial infections both at admission (positive cultures within the first 72hrs following admission) and hospital-acquired infections (positive cultures after 72hrs following admission) on mortality is explored. The manuscript was published in the Paediatr Int Child Health. 2016;Jul 4:1-8.

Chapter 5: *Tuberculosis in HIV-infected South African children with complicated severe acute malnutrition.*

A description of the frequency of clinically diagnosed and culture-confirmed mycobacterium Tuberculosis (MTB) infection in this cohort of severely malnourished HIV-infected children is presented together with a description of sampling techniques. Factors predicting culture-confirmed MTB using a regression analysis is explored. The manuscript was submitted to: International Journal of Tuberculosis and Lung Disease, IJTLD-10-16-0753 and is under review.

Chapter 6: *Malnutrition increases microbial translocation, systemic immune activation and immune exhaustion and impairs immune recovery in HIV-infected children.*

In this chapter the effect of malnutrition on microbial translocation, systemic immune activation and immune exhaustion is explored by comparing these factors in four cohorts

(HIV+/SAM+, HIV+/SAM-, HIV-/SAM+ and HIV-/SAM-). In the HIV-infected children, the effect of malnutrition on immune recovery at week 48 is described. The manuscript was submitted to AIDS Research and Human Retroviruses, #AID-2016-0261 and is under review.

This chapter was a collaborative effort, with the involvement of multiple different laboratories and partners who contributed. The individual author contributions have been declared in the Declaration (Pg iii/iv)

Chapter 7: *Synthesis*

In this chapter a synthesis of major findings of the previous chapters are presented. Recommendations arising from these findings and implications for future research are discussed.

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BRIDGING TEXT:

In the literature review, the optimal timing of ART initiation in severely malnourished children has been identified as a knowledge gap. In the following chapter the effects of initiating ART during the acute phase of malnutrition (early arm) compared with ART initiation after nutritional improvement (delayed arm), are explored in a randomized controlled trial with a 48-week follow-up period.

CHAPTER 2

HIV-INFECTED CHILDREN WITH SEVERE ACUTE MALNUTRITION: A RANDOMIZED CONTROLLED CLINICAL TRIAL COMPARING EARLY VERSUS DELAYED INITIATION OF ANTIRETROVIRAL TREATMENT

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2.1 Abstract

Background:

Delays in prompt HIV diagnosis and antiretroviral treatment (ART) initiation in children from low and middle-income countries frequently results in malnutrition at initial presentation. Despite ART initiation, HIV positive children with malnutrition have a higher mortality and delayed immune recovery. The optimal timing of ART initiation in children with malnutrition has not been established.

Methods:

Eighty-two HIV-infected children with severe acute malnutrition (SAM) admitted to King Edward VIII Hospital between July 2012 and December 2015 were enrolled. Patients were randomized to initiate ART within 14 days from admission (Early arm) or delay ART initiation until nutritional recovery and more than 14 days from admission (Delayed arm). All patients received a standardized treatment and feeding protocol and were evaluated at 4, 8, 12, 24 and 48 weeks.

Findings:

The average age of the patients at baseline was 23.3 months (SD 27.9, range 1.6–129 months). The mean time from admission to ART initiation was 5.6 days (SD 4.4) in the early arm and 23 days (SD 5.8) in the delayed arm ($p < 0.001$).

There was no significant difference in mortality ($p = 0.621$), virologic response ($p = 0.527$) and anthropometric response ($p = 0.566$) between the two groups at 48 weeks. However the rates of change in CD4, HIV viral load, Weight-for-age Z-score and Height-for-age Z-score occurred earlier and favored the delayed arm.

Interpretation:

The results of this study support delaying ART initiation to 14 days after starting nutritional rehabilitation in HIV-infected children admitted with SAM.

Keywords:

Severe Acute Malnutrition (SAM), Timing, ART Initiation

2.2 Introduction

Over 240,000 children under the age of 15 years were living with HIV in South Africa in 2015 (1). Ensuring early access to antiretroviral treatment (ART) is vitally important for

improving outcomes of these children; however only 34% of eligible children receive ART (2). This large gap in ART access is likely due to delays in HIV diagnosis and ART initiation, resulting in children presenting with more advanced clinical presentations such as malnutrition. Malnutrition therefore remains a common clinical syndrome at initial presentation in HIV-infected children from low and middle income countries (LAMIC) (3).

HIV-infected children with severe–acute malnutrition (SAM) represent a distinct clinical entity characterized by a complex interplay between increased energy expenditure with an increased basal metabolic rate, higher rates of co–infections, diarrhea and malabsorption, increased micronutrient deficiencies and higher rates of food insecurity and poverty (4). Management is complicated by gastro–intestinal side effects to antiretroviral drugs, perturbed lipid and glucose metabolism, and altered pharmacokinetics of ART and antibiotics (5).

ART initiation is further complicated by physiological changes during nutritional recovery in malnourished children. These changes include continuous shifts in the oxidative stressors, lean body mass and drug distribution, intestinal absorption of oral medications, serum albumin concentration for drug-binding, and the magnitude of mitochondrial, hepatic or renal dysfunction and re-feeding syndrome (6–7). The potential effect on the frequency of adverse effects and pharmacokinetics following ART initiation has not been previously addressed.

Despite nutritional rehabilitation, the CD4 counts in HIV–infected severely malnourished children continue to decline, and they have a three–fold higher probability of mortality compared to their non-infected counterparts (8,9). Although ART in HIV-infected children has significantly improved survival and quality of life, SAM remained an independent predictor of mortality even with appropriate immunologic and virologic responses to ART (10–14).

In 2010 the World Health Organization’s (WHO) Nutrition Guideline Advisory Group identified determining the optimal timing of ART initiation and ART dosing for HIV–infected children with SAM as a key issue for which guidance must be developed (15,16). A more recent systematic review found a paucity of quality data in this area and found no randomized controlled trials addressing antiretroviral treatment of HIV-infected children with SAM (17). Only a handful of observational studies have presented data on the nutritional status of children started on ART and helped to very indirectly inform the timing, dosing, and management of complications, such as immune reconstitution inflammatory syndrome (IRIS) in this group. We therefore conducted a prospective randomized controlled trial to evaluate and compare the virologic, immunologic and anthropometric responses of initiating ART before or after nutritional recovery in HIV-infected children admitted with SAM.

2.3 Patients and Methods

Patients were recruited from July 2012 to December 2015 from King Edward VIII Hospital, a referral hospital with a 100 bed paediatric unit in Durban, South Africa. All ARV

naïve HIV positive children admitted with SAM were eligible for inclusion in the Malnutrition and Antiretroviral Timing in Children with HIV (MATCH) trial (Clinical trial registry number: PACTR 21609001751384).

SAM was defined as per the WHO definition i.e. a Weight-for-length more than 3 z-scores below the median, Mid-upper arm circumference (MUAC) < 115 mm or peripheral oedema (18). HIV was diagnosed as per the South African National HIV Treatment Guidelines (19). In children less than 18 months, two HIV polymerase chain reactions (PCR) were performed (Taqman HIV-1 Qualitative Test (Roche Molecular Systems, Inc.), while in children over 18 months two HIV rapid tests or an enzyme-linked immunosorbent assays was performed.

All patients were managed as per the standardised hospital policy based on the WHO Guidelines for the In-patient Treatment of Severely Malnourished children with standardized re-feeding guidelines (18). Medical management of patients was at the discretion of the treating clinician.

Patients were randomized to either initiate ART within 14 days from admission (Early arm) or delay ART initiation (Delayed arm) until nutritional recovery (as defined below) and more than 14 days from admission. Patients achieved nutrition recovery when they reached a weight for height z-score (WHZ) of -2 , achieved at least 15% weight gain or demonstrated resolution of edema and return of the patient's appetite. A pre-determined computer generated randomization table was used, with randomization blocks of 10 weighted to ensure equal numbers of patients with tuberculosis (TB) in each arm.

Antiretroviral treatment was as per the South African National ART treatment guidelines (2012–2015) (19). Children less than three years were started on Abacavir, Lamivudine and Lopinavir/ritonavir while children over three years were started on Abacavir, Lamivudine and Efavirenz. Drug doses were as per country specific guidelines (based on the WHO weight band dosage charts). Patients requiring Rifampicin and Lopinavir/ritonavir had additional ritonavir prescribed as per the weight band dosage table (19).

Patients were followed up at four, eight, 12, 24 and 48 weeks following admission. Clinical assessment included a clinical examination for adverse effects and anthropometric evaluations (weight, height, MUAC and skin fold thickness over two sites: triceps, subscapular). At each study visit patients had an HIV viral load, CD4 count, Full Blood Count, Liver Function test, Urea and Electrolytes, Triglycerides/Cholesterol and Blood Glucose performed. Virologic treatment response was defined as a HIV viral load <1000 copies/ml at 48 weeks. Immunologic treatment response was defined as an improved CD4 count to WHO Immune stage one and two (20). Anthropometric treatment response was defined as body mass index Z-score (BMIZ) or WHZ score of greater than -1 below the mean. Patients were clinically assessed at each study visit for features of an Immune Reconstitution Inflammatory Syndrome (IRIS) event (a new onset or worsening of a condition associated with ≥ 1 log drop in HIV viral load as defined by

the IRIS diagnostic criteria by French) (21). All laboratory results were graded using the DAIDS Grading tables (22) and Grade three and four laboratory results were managed according to the South African National ART treatment guidelines (19).

Written informed consent was obtained from the caregivers of all children enrolled in the MATCH study. The trial was approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal and King Edward VIII Hospital. The study was supported in part by grant number: R24TW008863 from the Office of the U.S. Global AIDS Coordinator and the U. S. Department of Health and Human Services, National Institutes of Health (NIH OAR and NIH ORWH).

The sample size was calculated using SAS Version 9.2 to detect a difference in nutritional recovery between the two arms (mean difference of 0.5 Z-score in WHZ) to achieve a power of 80% and a significance of 5% (23).

Data were analysed using Stata 13.0 (StataCorp 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). The student T-Test was used to compare the means and standard deviations between the two arms. Analyses were performed as per intention-to-treat. Significant time point comparisons of categorical endpoints by trial arm were assessed using the Fishers exact test. A mixed effects linear model was employed to assess change in continuous markers over time by trial arm. A random effects logistic regression was used to assess dichotomous endpoints (e.g. response) by trial arm. An adjusted p-value of <0.05 was considered statistically significant.

2.4 Results

A total of 82 Black African patients were enrolled in the study, 40 in the early arm and 42 in the delayed arm. At week 48, in the early treatment arm 27 patients (68%) completed follow-up (six deaths and seven lost to follow-up (LTFU)) while in the delayed arm 31 patients (74%) completed follow-up (eight deaths, three LTFU) (Figure 5).

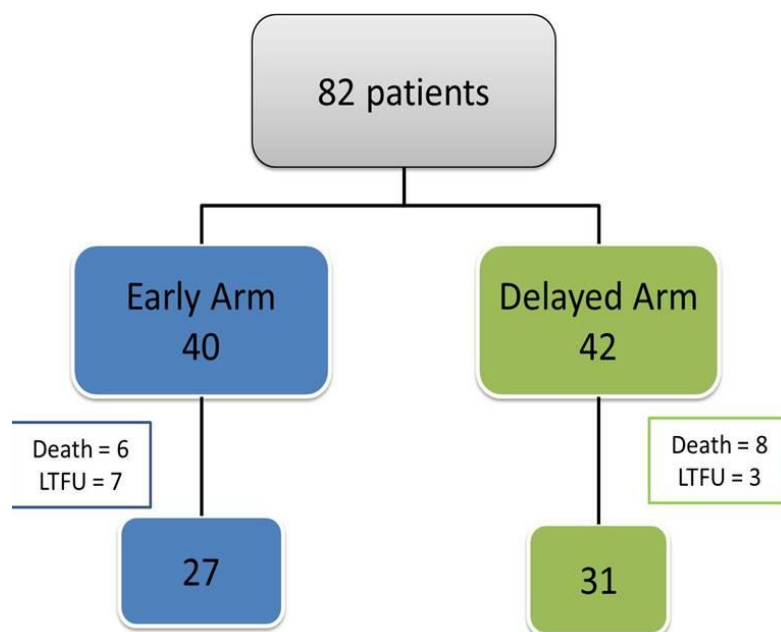


Figure 5. Study Schema over 48 weeks

Caregivers of patients who defaulted the week 48 visit were contacted telephonically. Of the seven patients who were LTFU in the early arm, only two caregivers were contactable and reported that the child was alive, in-care but relocated to another city. Two of the three patients who were LTFU in the delayed arm were contacted. Both patients were alive and in-care at another facility. The reason for relocating was to return to family homes in neighboring provinces or countries. Fewer patients were LTFU in the delayed arm as compared to the early arm, however this difference was not statistically significant (17.5% vs. 7.1%, $p=0.189$).

The average age of the patients at baseline was 23.3 months (SD 27.9, range 1.6–129 months) with a slight male preponderance ($n=47.58\%$). The majority (78%) of the patients had low CD4 counts (WHO immune stage 3 or 4) and 58% were classified severely immune-compromised (WHO immune stage 4). Lopinavir/ritonavir based ART was the most common regimen with 87.8% ($n=35$) and 88% ($n=37$) of subjects on this regimen in the early and delayed arms respectively.

Comparison of patient characteristics by treatment arm at baseline revealed no significant differences apart from time from admission to ART initiation ($p<0.001$) i.e. mean of 5.6 days (SD 4.4) in the early arm and 23 days (SD 5.8) in the delayed arm. (Table 8).

Table 8. Patient Characteristics at baseline (at study entry/admission)

	Early N (SD)	Delayed N (SD)	p value
Total number (82)	40	42	
Age (months)	25.7 (1.7–129)	21.1 (1.6–99.7)	0.64
Gender (M:F)	60% (24): 40% (16)	55% (23): 45% (19)	0.52
CD4 Abs (mean)	805 (800)	952 (745)	0.59
CD4 % (mean)	16% (4.5)	18% (8)	
HIV Viral load (copies/mL)	1893372 (3125480)	2774529 (2534080)	0.27
ABC/3TC/EFV	12% (10)	12% (5)	0.35
ABC/3TC/LPV/rvtv	88% (30)	88% (37)	
Time to ART initiation (days)	5.6 (4.5)	23 (5.8)	0.0001
Tuberculosis at baseline	38% (16)	35.7% (15)	0.49
WAZ (mean)	-3.5 (1.2)	-3.4 (1.6)	0.54
WHZ (mean)	-2.2 (1.6)	-2.2 (2.2)	0.36
MUAC (mean)	11.4 (1.6)	11.7 (2.1)	0.66
Hb (admission)	8.9 (2.1)	8.8 (2.1)	0.37
TP (mean)	66 (17.7)	65 (16.6)	0.44
Alb (mean)	22.5 (8.3)	23.8 (6.9)	0.66

2.4.1 Mortality

During the 48-week study period 14 patients (17%) demised, six (15%) in the early arm and eight (19%) in the delayed arm. The difference in the mortality between the two arms was not statistically significant ($p=0.626$). The average time from enrollment to death was 74.4 days (SD: 59.9), range 12–252 days. The mean time to death was 92.7 days in the early arm compared with 60.8 days in the delayed arm. No patient demised prior to ART initiation. The majority (78.6%) of deaths occurred before 12 weeks, 67% and 87.5% in the early and delayed arms respectively.

Adverse Effects/IRIS

A total of 23 IRIS events were documented in 21 (25%) patients (Two patients had both TB and BCG IRIS), 13 (56.5%) in the early arm and 10 (43.5%) in the delayed arm ($p=0.381$). Five patients (21.7%) developed unmasking TB IRIS during the study period, two in the early arm and three in the delayed arm, while seven patients (30.4%) developed BCG IRIS (all with local BCG scar ulceration with regional lymphadenitis), four in the early arm and three in the delayed arm. The remaining IRIS events were dermatological manifestations, three patients with seborrheic dermatitis, three patients with candida skin rashes and one patient each with

Herpes stomatitis, planar warts, vulval warts, molluscum contagiosum and **tinea corporis**. None of the IRIS events required discontinuation of ART or resulted in mortality.

Following discharge from hospital after study entry, 14 patients required readmission to hospital, five (12.5%) in the early arm and nine (21.4%) in the delayed arm. Majority (64%) of re-admissions were due to lower respiratory tract infections followed by acute gastroenteritis (21%). Sixty four percent of re-admissions occurred within the first 12 weeks following study entry.

None of the patients required alteration or discontinuation of ART due to a graded laboratory abnormality. Table 9 provides a summary of DAIDS grade three and four laboratory investigations at entry, week 24 and week 48.

Table 9. Summary table of Grade 3 and 4 Laboratory toxicities at baseline, 24 weeks and 48 weeks

		Hb	Platelets	Total Bili	ALT	GGT
Grade 3	Baseline	5	4	1	1	3
	24 wks	2			3	2
	48 wks			1		
Grade 4	Baseline	9	1			4
	24 wks					2
	48 wks			1		

Hb: Haemoglobin, Total Bili: Total **Bilirubin**, ALT: Alanine Transaminase, GGT: Gamma-glutamyl transpeptidase

2.4.2 CD4 Count

At 48 weeks, 58.5% of patients were WHO immune stage one, 19.5% stage two, 12.2% stage three and 9.8% stage four compared to 16%, 10.7%, 28.6% and 44.6% at baseline.

There was a significant increase in the overall mean CD4 count from a baseline count of 878.5 cells/uL (SD 772.8) to 1447.9 cells/uL (SD 739.0) at 48 weeks (p-value=0.001). The mean CD4 count at week 48 was 1366.7 (SD 732.4) cells/uL in the early arm and 1518.2 (749.8) cells/uL in the delayed arm (p=0.532)

Comparison of the change in CD4 count using a multiple mixed effects linear regression model (Figure 6) suggested no statistically significant difference in immune recovery between the two groups. However the delayed arm did have a higher mean CD4 (more rapid gain) by week 12 compared to the early arm with this difference narrowing towards 48 weeks. This difference at 12 and 24 weeks was not statistically significant (p=0.433 and 0.436 respectively).

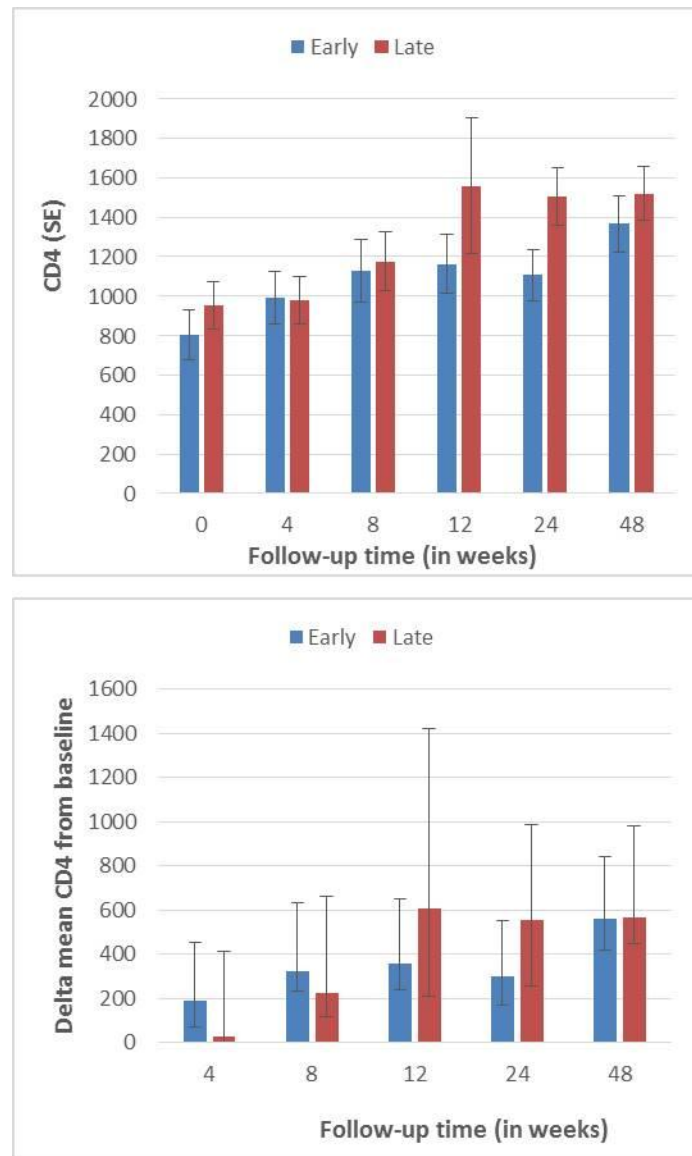


Figure 6. (a) Mean CD4 and (b) delta mean CD4 from baseline by trial arm and time point

2.4.3 Viral Load

At 24 weeks, 67.3% 18 of combined patients achieved protocol define response (VL <1000 copies/ml) overall, 59.7% 11 in the early arm compared to 75% 7 in the delayed arm, but this difference was not statistically significant. By 48 weeks the number of patients with protocol defined virologic response increased to 81.1% overall 11, 77.8% 6 in the early arm and 84% 5 in the delayed arm. This difference at 48 weeks was not statistically significant before and after multivariable adjustment (OR=1.66, p=0.527, 95% CI 0.34–8.06). (Table 10).

Table 10. Comparison of (a) primary endpoints at 48 weeks (b) anthropometric change from baseline to 48 weeks by trial arm

Endpoint	Variable	Odds Ratio*	95% CI	p-value
Anthropometric response at 48 weeks	Late vs early (ref)	1.66	0.29–9.31	0.566
Virologic response at 24 weeks	Late vs early (ref)	2.04	0.52–7.98	0.305
Virologic response at 48 weeks	Late vs early (ref)	1.66	0.34–8.06	0.527
Mortality at 48 weeks	Late vs early (ref)	1.38	0.38–4.96	0.621
Favourable outcome at 48 weeks	Late vs early (ref)	1.42	0.49–4.12	0.52
*Adjusted for age, gender, WHO immune stage at baseline, Tuberculosis, Hb at baseline, TP at baseline, Alb at baseline				

Endpoint	Variable (change at 48 weeks versus baseline)	Coef*	95% CI	p-value
HAZ	Late vs early (ref)	+0.90	-0.04 to 1.85	0.061
BMI for age Z	Late vs early (ref)	+0.08	-0.95 to 1.10	0.884
WHZ	Late vs early (ref)	+0.25	-0.66 to 1.17	0.583
WAZ	Late vs early (ref)	+0.49	-0.22 to 1.19	0.176
*Adjusted for age, gender, WHO immune stage at baseline, Tuberculosis, Hb at baseline, TP at baseline, Alb at baseline				

Using a stricter definition of virologic response (VL < 400 copies/ml), 77.9% of patients achieved virologic response at 48 weeks, 83.9% in the delayed arm and 70.37% in the early arm, again this difference was not statistically significant (OR= 2.02, p= 0.231, 95% CI 0.64–6.36).

A multiple mixed effects linear regression model evaluating viral load endpoint and change therein at weeks four, eight, 12, 24 and 48, showed an appropriate delay in viral load reduction in the delayed arm compared to the early arm at week four (+1.57 mean log VL in delayed arm, p<0.001) and eight (+0.79 mean log VL in delayed arm, p=0.098) (Figure 7). At week 12 the log VL fall was equivalent (+0.22 mean log VL in delayed arm, p=0.625, 95% CI -0.66 to 1.1) in both arms, however by week 24 the delayed arm had a statistically significant higher drop in log VL compared to the early arm (-1.06 mean log VL in delayed arm, p=0.018, 95% CI -1.94 to -0.18). This difference persisted to week 48, and remained statistically significant (-0.99 mean log VL in delayed arm, p=0.025, CI -1.85 to -0.12).

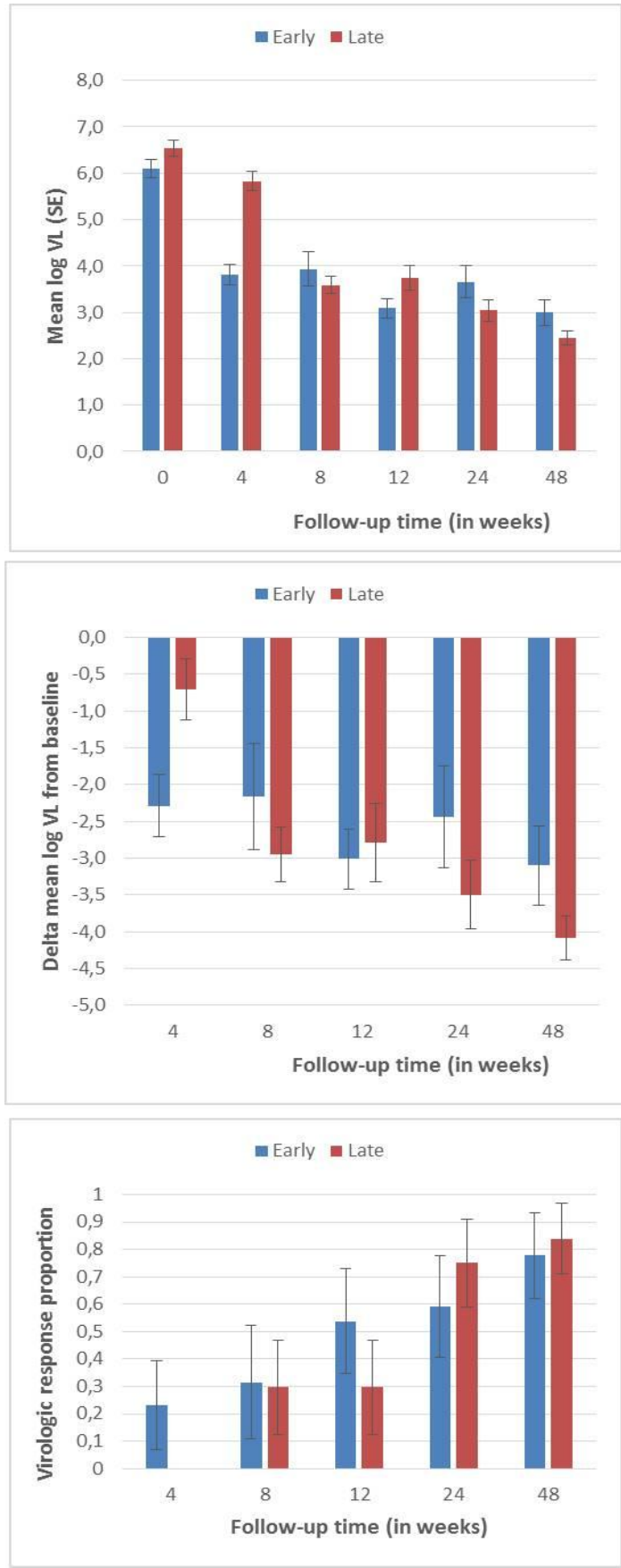


Figure 7. (a) Mean log VL, (b) delta mean VL from baseline and (c) virologic response proportion by trial arm and time point

2.4.4 Anthropometry

At week 48, 76.6% of patients achieved an adequate anthropometric response, with similar responses in both arms (77.2% in early arm and 76.6% in the delayed arm ($p=0.918$)). The adjusted comparison of anthropometric response at week 48 was not significant (OR=1.66, $p=0.566$, 95% CI 0.29–9.31)

There were no significant differences in BMIZ or WHZ scores between the two arms at 48 weeks (OR=0.79, $p=0.43$, 95% CI –0.96–2.25). At week 48 the difference in weight for age Z-score (WAZ) between the two arms was marginally significant (OR=1.67, $p=0.094$, 95% CI 0.11–1.44) whilst the difference in height for age Z-score (HAZ) was statistically significant (OR=2.51, $p=0.012$, 95% CI 0.27–2.25). (Figure 7) After adjusting for age, gender, immune stage, tuberculosis, haemoglobin (Hb), total protein (TP) and albumin (Alb), only the change in HAZ remained marginally significant. (Figure 8).

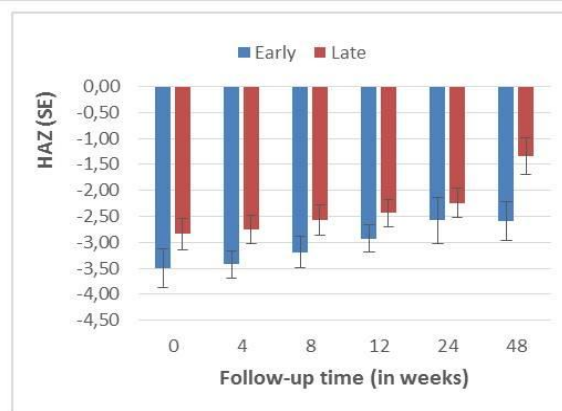
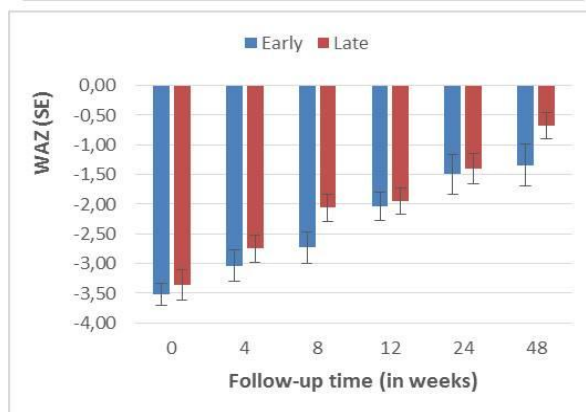
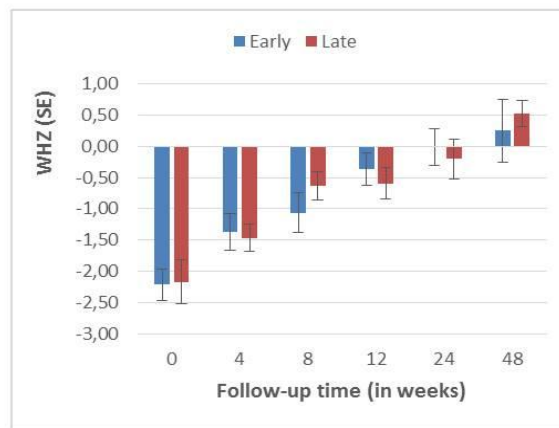
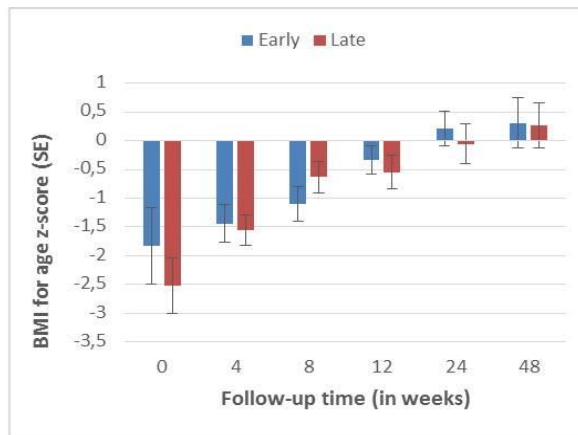


Figure 8. (a) Mean BMI for age z-score, (b) Mean Height-for-age z-score (HAZ), (c) Mean Weight-for-age (WAZ) z-score and (d) mean Weight-for-height z-score (WHZ) by trial arm and time point

2.5 Discussion

This prospective randomized controlled trial comparing early versus delayed ART initiation in a cohort of HIV-infected children, admitted with severe acute malnutrition has demonstrated no significant differences in immunologic, virologic or anthropometric treatment responses at 48 weeks. However significantly improved rates of change in the viral load, WAZ and HAZ favored the delayed arm.

The WHO Consolidated ART guidelines have, since 2013, recommended that all children less than five years of age initiate ART on diagnosis irrespective of WHO clinical stage or CD4 count. This was extended to all children, adolescents and adults in 2016 (24).⁴ Despite this “test and treat” approach to ART management in young children, ART initiation targets in children have lagged behind adult targets. Delayed HIV diagnosis and treatment, together with the rapid clinical deterioration of HIV-infected children has resulted in children from resource constrained settings frequently being malnourished on diagnosis of HIV infection. The question of the optimal timing of ART initiation in malnourished children has thus far not been addressed in a randomized controlled trial.

In a Malawian retrospective observational study of children with uncomplicated malnutrition receiving outpatient therapeutic feeding, ART initiation within 21 days had a higher rate of nutritional recovery (86% vs. 60% $p < 0.01$) and higher rate of weight gain (3.6 vs. 1.6g/kg/day) when compared to those who had delayed ART initiation (25). In contrast, in unpublished retrospective analysis of 345 HIV-infected Ugandan children, where 21% of the cohort were moderate or severely malnourished, mortality in children who initiated ART within ten weeks of diagnosis was higher compared to those starting ART later after adjustment for age, sex, CD4%, and WHO clinical stage (OR: 2.8, 95% CI: 1.0-33-5.9, $P=0.007$) (17). A randomized controlled trial comparing urgent versus post-stabilization ART initiation in Kenyan hospitalized children (32% were malnourished) showed no difference in mortality at 24 weeks (26). No study reported on the timing of the introduction of ART with respect to that of nutritional rehabilitation for SAM (27).

In a systematic review of mortality associated with SAM in sub-Saharan Africa, HIV positive children had a significantly higher mortality compared to HIV negative children (30.4% vs. 8.4%) during rehabilitation (28). Few studies have reported specifically on mortality in HIV positive children with SAM beyond the rehabilitation phase (29). In the MATCH cohort, the mortality at 48 weeks and the inpatient mortality rate was far lower than the reported mortality. The in-patient mortality was in line with the expected mortality in a well-resourced tertiary hospital, where patients were management in a high-care setting. No significant difference was demonstrated between the two treatment arms, although the study was not powered to detect differences in mortality. The majority of the mortality (78.6%) and hospitalizations (64%) occurred in the first three months following study entry, which is similar

to other studies. Potential strategies to decrease these events, such as more frequent clinical assessments, education of caregivers of danger signs and the need for urgent review at a health care facility and opportunistic infection prophylaxis may be of benefit. In the REALITY trial, severely immune-compromised HIV positive **adult and children** initiating ART with a regimen of enhanced opportunistic infection (OI) prophylaxis compared to standard prophylaxis demonstrated decreased mortality, new TB, cryptococcal or candida infections and hospitalizations (30). Further studies evaluating enhanced OI prophylaxis should be considered in HIV-infected children with SAM and other high-risk criteria at ART initiation.

IRIS is a common clinical entity in children initiating ART in LAMIC, with a prevalence in the literature ranging from 20–38% which is similar to the incidence reported in this study (21,31). TB IRIS was the most frequent presentation in a study of HIV-infected children in Uganda, (29%). Time on ART and pre ART CD4 count were significantly associated with IRIS events however malnutrition (WHZ <-2SD) was not associated with increased incidence of IRIS in the same study (32). The absence of a significant difference in the frequency of TB IRIS between the arms could be due to the high proportion (36.9%) of patients who were initiated on TB treatment. In the current study, BCG IRIS was the most frequent IRIS event, which was not reported in the studies of Ugandan and Thai children, **however BCG IRIS has been reported in cohorts South African children initiating ART** (31,33–35). The presence of high incidence of BCG IRIS may be related to the timing of vaccination and strain of BCG used in South Africa.

Once initiated on ART, maintaining patients in care is vitally important in order to achieve the WHO goal of 90% virologic suppression in patients initiated on ART (36). In this study all patients received the same package of counseling sessions, one post-test counseling session and three adherence counseling sessions (37) and counselors were unaware of the randomization of the patients. Patients in the early arm were started on ART before the counseling sessions were complete. The psychological impact of a caregiver being informed of a child's HIV status concurrently with being counseled regarding the need to administer ART, may account for the difference in numbers of LTFU between the two arms although this difference was not significant. Caregivers may require time to first assimilate and accept the diagnosis of HIV before being able to fully understand and accept the adherence counseling sessions.

Data on virologic responses in children with SAM are limited due to limited access to routine viral load testing in settings where SAM is common. Several studies have reported on virologic responses in sub-Saharan Africa and included malnourished children. In the PROMOTE trial, virologic response (VL<400c/ml) at 48 weeks was 80% and 76% for a Lopinavir/ritonavir based regimen and Efavirenz based regimens respectively (38). Although the difference in virologic response did not reach statistical significance in our study, the multiple mixed effects linear regression model revealed a significant difference favouring the

delayed arm at week 24 and week 48. The model did confirm the delay in viral load decline in the delayed arm at week four due to the delay in ART initiation. Altered pharmacokinetics of antiretroviral drugs in patients initiating ART during the acute phase of nutritional recovery may account for the differences in the viral load change, significantly favouring the delayed arm at week 24 and 48. A population pharmacokinetic study conducted during this study and an ongoing IMPAACT study may address this important research question.

Several studies from sub-Saharan Africa have documented good immunologic and growth responses in HIV-infected children initiated on antiretroviral treatment. However children with SAM have impaired immunologic recovery compared with well-nourished children (8,9). Although the difference in immunologic recovery between the arms was not statistically significant in our study, the increase in the CD4 count occurred earlier in the delayed arm. Delaying ART initiation may allow the gut microbiota time to normalize, decreasing microbial translocation and immune activation, thus facilitating more rapid immune recovery (39,40).

Similarly the better WAZ and HAZ scores may suggest an improved gut recovery and absorptive capacity in the delayed arm, possibly mediated by changes in the gut microbiota. Future studies are required to explore this finding and develop potential adjunctive treatment strategies to improve immune recovery and growth.

There are several limitations to the study, including that the small sample size was not powered to detect smaller differences between the study arms. Mortality and LTFU, further limited the ability of the study to detect difference at 48 weeks.

2.6 Conclusion

HIV-infected children admitted with SAM and initiated on ART demonstrated significant improvements in CD4 counts and anthropometric parameters, together with significant viral load reduction compared to baseline. In this randomized controlled trial comparing early versus delayed ART initiation in HIV-infected children admitted with SAM, although the differences in CD4 count, viral suppression and anthropometric response at 48 weeks was not significant, the rates of change in CD4, viral load, WAZ and HAZ scores occurred earlier and favored the delayed arm. Based on the results of this study, we recommend that ART initiation in children with SAM should be delayed for at least two weeks after starting nutritional rehabilitation.

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BRIDGING TEXT:

Delaying ART initiation to after nutritional recovery resulted in improved rates in change of WAZ, HAZ and viral load compared to ART initiation during the acute phase of malnutrition. A potential biological mechanism explaining this observation is the alteration of antiretroviral drug pharmacokinetics in malnourished children. In the literature review, the absence of data describing lopinavir pharmacokinetics in severely malnourished children specifically and the limited data describing lopinavir pharmacokinetics in malnourished children in general was described. In the following chapter, the pharmacokinetics of lopinavir is explored in severely malnourished children by developing a model to describe the observed lopinavir levels and explore the relationship of this model with patient and treatment characteristics.

CHAPTER 3

POPULATION PHARMACOKINETICS OF LOPINAVIR IN SEVERELY MALNOURISHED HIV INFECTED CHILDREN AND THE EFFECT ON TREATMENT OUTCOMES

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Keywords: Population Pharmacokinetics, Lopinavir, Severe acute malnutrition, Treatment outcomes

3.1 Abstract

Background: In developing countries, malnutrition remains a common clinical syndrome at antiretroviral treatment (ART) initiation. Physiological changes due to malnutrition and during nutritional recovery could affect the pharmacokinetics of antiretroviral drugs.

Methods: HIV-infected children admitted with severe acute malnutrition were randomised to early or delayed initiation of lopinavir/ritonavir, abacavir and lamivudine using WHO weight-band dosage charts. Lopinavir concentrations were measured on day 1 and day 14. Thereafter patients were followed-up to week 48. The population pharmacokinetics of lopinavir was described using NONMEM v7.3. Covariates were screened to assess their influence on the pharmacokinetics of lopinavir and the relationship between pharmacokinetic variability and treatment outcomes was assessed.

Results: 502 lopinavir concentrations were collected from 62 paediatric patients aged 1.6-78 months (median: 12 months). Rifampin-based antituberculosis treatment and “super-boosted” lopinavir/ritonavir was prescribed in 20 patients. Lopinavir disposition was well described by a one-compartment model with first order elimination. Neither randomisation to early or delayed ART, tuberculosis co-medications nor anthropometrical measurements explained the pharmacokinetic variability. Allometrically scaled fat-free mass (FFM) influenced apparent clearance ($CL/F, 3.1L/h/5.6kg$) and volume of distribution ($V_d/F, 9.6L/5.6kg$). Pharmacokinetic exposure did not correlate with virologic outcomes or death at 12 or 48 weeks.

Conclusions: Lopinavir pharmacokinetics was influenced by FFM and not by timing of ART initiation or tuberculosis co-medication in severely malnourished HIV-infected children. Lopinavir pharmacokinetics was found to be highly variable and

bioavailability greatly reduced, resulting in a high CL estimate in this population. The role of lopinavir dose adjustment should be further evaluated in severely malnourished children initiating ART.

3.2 Introduction

Malnutrition is a common clinical feature at initial Human Immunodeficiency Virus (HIV) diagnosis in sub-Saharan African children (1,2) and is a significant risk factor for mortality (2). The causes of malnutrition in this setting are multifactorial including delays in HIV diagnosis and antiretroviral treatment (ART) with resultant prolonged viraemia with an increased energy expenditure and basal metabolic rate together with higher rates of opportunistic co-infections, diarrhoea, malabsorption, food insecurity and poverty (3,4).

Immunologic and virologic responses together with the mortality (5,6) of severely malnourished HIV-infected children are higher compared to their non-malnourished counterparts despite nutritional rehabilitation and ART in HIV-infected children (7,8).

The cause of this difference has not been determined, and may be caused by altered pharmacokinetics of antiretroviral medications in malnourished children. Alternatively food insecurity may be a surrogate marker of an unstable social environment and poor adherence, resulting in poorer outcomes (9).

The physiologic characteristics of malnutrition and changes following nutritional recovery are particularly dynamic due to shifts in oxidative stressors, areas of lean body mass, serum albumin levels, intestinal changes and degrees of mitochondrial, hepatic or renal dysfunction (10,11). These physiological characteristics can potentially affect the pharmacokinetics of medications in malnourished children resulting in increased adverse events due to supra-therapeutic drug levels. Alternatively, resulting in prolonged exposure to sub-therapeutic antiretroviral drug levels, which is a major contributor to the evolution of HIV drug resistance (12).

There is a paucity of data evaluating the pharmacokinetics of antiretroviral drugs in malnourished children. The most extensively studied antiretroviral drug in malnourished children is nevirapine, which is no longer the preferred agent for treatment of ART naïve children in current WHO guidelines (13). Malnutrition had no effect on plasma total or unbound nevirapine exposures in Malawian children, of whom 32% had mild to moderate malnutrition (13). Other studies found lower nevirapine concentrations in stunted children compared with non-stunted children (12,14). Most

recently, it was reported that efavirenz and lopinavir (LPV) exposures were reduced in Ugandan children, 48% of whom were malnourished, compared to historical data from children in resource-rich countries (15).

Overall, the impact of severe malnutrition on the pharmacokinetics of antiretroviral drugs is difficult to predict as drug absorption and elimination is decreased in malnutrition resulting in opposing effects (10). There is no data evaluating the use of different ART regimens or strategies in severely malnourished HIV-infected children. This study aimed to determine the effects of nutritional rehabilitation on LPV pharmacokinetics in severely malnourished HIV-infected children and to explore the relationship between LPV pharmacokinetic exposure and virologic outcomes in this study cohort.

3.3 Methods

3.3.1 Patients and Data

Between June 2012 and December 2015, HIV infected infants and children admitted to King Edward VIII Hospital, Durban, South Africa, with severe acute malnutrition (weight for height below -3 z score, mid-upper arm circumference <11.5cm or oedema) was enrolled in the MATCH (**M**alnutrition and **A**RT **T**iming in **C**hildren with **H**IV) Study (Clinical trial registry number: PACTR 21609001751384).

Patients were randomized to either initiate ART within 14 days from admission to the hospital (early arm) or delay ART initiation until after 14 days from admission or when nutritional improvement was demonstrated (delayed arm). Patients demonstrated nutritional improvement when they reached a weight for height z-score (WHZ) of -2, achieved at least 15% weight gain or demonstrated resolution of oedema and return of their appetite. **Apart from the timing of ART, all other management was as per the standard of care.** Antiretroviral treatment was administered as per the South African National ART guidelines (2012-2015) (16). Children less than three years or less than 10kgs were started on abacavir, lamivudine and lopinavir/ritonavir (LPV/rtv). Drug doses were according to country specific guidelines and WHO weight band-based dosage charts. Patients requiring rifampicin containing anti-tuberculosis treatment received “super-boosted” LPV/rtv (LPV:rtv ratio of 1:1) as per the weight band dosage table (16). Adherence to medication during hospitalization was verified by review of the hospital prescription chart.

Blood samples were drawn on the first day of ART initiation and day 14 post ART initiation according to the following sampling schedules; on day 1, samples were drawn at the following nominal time points: 1.3 - 1.8 hrs. post dose, 3 – 4 hrs. post dose, 5 - 7 hrs. post dose and 8 – 10 hrs post dose. On day 14, one sample was drawn 30 minutes prior to dosing, and 1.3 - 1.8 hrs. post dose, 3 – 4 hrs. post dose, 5 - 7 hrs. post dose and 8 - 10 hrs. post dose. Patients remained inpatients between Day 1 and Day 14. Whole blood was transported to the laboratory on ice within an hour of being drawn and centrifuged at 2 000 rpm for 10 minutes using a refrigerated centrifuge. Plasma (500uL) was aliquoted into cryotubes and stored at -70 °C before being shipped on dry ice for measurement of drug concentrations.

HIV viral loads (Cobas Ampliprep/ Cobas TaqMan system supplied by Roche) were measured at 12 and 48 weeks following study entry. Treatment failure was defined according to virological status, where VL >1000 copies/mL or death was considered treatment failures and VL <1000 copies/mL was defined as treatment success.

3.3.2 Assay

LPV assay: Samples were analysed for LPV using a validated liquid chromatography-mass spectrometry method as described previously (17). The lower Limit of Quantification (LoQ) for LPV was 0.0195ug/mL.

3.3.3 Pharmacokinetic Modeling

The population modeling was conducted using NONMEM® version 7.3 [19], Intel FORTRAN compiler and PsN® version 4.1 [20]. Structural model parameter estimates, inter-individual variability (IIV) and residual unexplained variability (RUV) were obtained by first-order conditional estimation with interaction (FOCE+I). The pharmacokinetic structural base model for LPV was initially explored followed by stochastic model evaluation, covariate model development and model evaluation steps. The IIV was modeled exponentially and inter-occasion variability (IOV) was modeled by an additional random effects parameter, as described previously (18) where P_{ij} represents the estimate of a parameter P for subject i on occasion j about the typical population value (P_{pop}). Parameter i,P is a random variable distributed with a mean value of 0 and variance of σ_P^2 which represents the IIV variability of P in the population. Parameter K is a random variable, was assumed to be sampled from a normal distribution of mean value 0 and a variance of π^2 , representing the variability of P on different occasions. An occasion was defined as a dose followed by at least one

observation. Here the maximum possible number occasions per patient was three, (i) day 1 observations grouped together, (ii) day 13 trough observation and (iii) day 14 observations grouped together. The RUV was estimated using proportional, additive and combined error models. First LPV concentration reported as below LoQ values within a dosing interval was set to a value of $\frac{1}{2}$ LoQ, others discarded.

Covariates screened to assess their influence on the pharmacokinetics of lopinavir included: early versus delayed ART initiation, study day, time since LPV start, weight, fat-free mass (FFM) (19) age, cholesterol, triglyceride, anthropometrical measurements, and the combined effect of rifampicin and extra ritonavir (in children on tuberculosis treatment and “super-boosted” LPV/rvt). Covariates were added and retained in the model when they: improved the fit of the model to the data; if biologically plausible, and; if a significant decrease in the objective function value (OFV) generated by NONMEM was noted. For nested models, the difference between a pair of OFV values when a covariate was included (full model), then excluded (reduced model), approximates to the Chi-square (X^2) statistic which can be tested for significance ($X^2_{1, 0.05} = 3.84$). Covariates representing continuous data items were screened separately using linear, power and exponential functions in which the parameterization was centered on a standard covariate value. For model evaluation diagnostics goodness-of-fit (GOF) plots and prediction- and variance corrected visual predictive checks (pred-var VPC) were used. The percentile bootstrap 95% confidence intervals around the final population model parameters were obtained using an automated nonparametric bootstrap with sample replacement (n=500 runs).

3.3.4 Treatment Outcomes versus Pharmacokinetics

Treatment outcomes were related to LPV concentrations on each study day (maximum 3 days/patient), by comparing patients with any sample below the Limit of Quantification (LoQ) ($< 0.0195\text{ug/mL}$) versus patients without any sample below the LoQ using a Chi-squared test, comparing the proportions.

Individual estimates of apparent LPV clearance (CL/F) and LPV exposure ($\text{AUC}_{0-12\text{ h}}\cdot\text{mg/L}$) for all study days were compared against treatment success and failure at 12 and 48 weeks using a one-way analysis of variance (ANOVA) test followed by Tukey’s test for post hoc analysis using RStudio (Version 0.99.484) with $p < 0.01$ considered as statistically significant.

3.4 Results

3.4.1 Patients and Data

A total of 62 patients had samples performed on day 1. Four patients demised and two were transferred between day 1 and day 14, resulting in 56 patients having samples drawn on Day 14. A total number of 502 LPV concentrations (8% of them reported as below LoQ, half of them were discarded) were available for analysis. Treatment outcomes were available for 37 patients at week 12 and 50 patients at week 48. Table 11 summarizes the patient characteristics of patients for the early and the delayed ART start groups.

Table 11. Demographic and pharmacokinetic data of the study population at baseline

	Early (mean±SD)	Delayed (mean±SD)	p-value
Patients assessed on day1:day14	34:31	29:27	-
Age (months)	15.5 (16.3)	14.59 (10.8)	0.57
Sex (M:F)	19:15	17:12	0.52
Oedema at admission (N)	5	7	0.65
Rifampicin co-administration (N)	10	10	0.19
Time to ART initiation (days)	6.2	23.7	0.0001
Weight for Age Z-score	-3.6 (1.2)	-3.2 (1.6)	0.12
Weight (kg)	6.5 (2.8)	6.6 (2.6)	0.86
Height for age Z-score	-3.6 (1.7)	2.9 (1.5)	0.08
BMI Z-score	-2.5 (1.8)	-1.8 (2.0)	0.15
Fat free mass (FFM)	5.1 (1.8)	5.5 (1.9)	0.41
Mid-upper arm circumference (cm)	11.1 (1.7)	19.0 (2.3)	0.15
HIV Viral load (copies/mL)	573134 (1709192)	1444639 (1897897)	0.14
Haemoglobin (g/dL)	8.9 (2.1)	8.8 (1.9)	0.74
Total Protein (g/dL)	65.0 (17.8)	65.2 (16.5)	0.99
Albumin (g/dL)	22.7 (8.0)	21.6 (6.4)	0.34
Creatinine	33.9 (34.2)	35.4 (31.6)	0.87
Cholesterol	2.7 (1.2)	2.9 (1.1)	0.48
Triglyceride	3.2 (2.4)	2.3 (1.5)	0.28

SD: Standard deviation, M: Male, F: Female, N: number, ART: antiretroviral treatment, BMI: Body mass index

3.4.2 Lopinavir Pharmacokinetics

The time-course of LPV disposition was well described by a one-compartment model with first order elimination. **Typical population parameter estimates (BOV**

(%CV)) were CL/F (L/h/5.6kg): 3.1 (126%), apparent volume of distribution (V_d/F , L/5.6kg): 9.6 and absorption rate (k_a , h^{-1}): 0.385 (56.8%). Using IOV to estimate variability on CL/F and k_a was superior to IIV. Estimation of IIV for the relative bioavailability (F; IIV= 69.5%) resulted in model improvement, with the individual estimates of F being constrained between 0 and 1 using logit- transformation. The proportional RUV (%CV) was 37.7% for samples taken within the first 5 hours after the dose and 27.2% with a BSV of 15.5%, allowing the RUV magnitude to vary from patient to patient (20). Final parameter estimates are shown in Table 12.

Table 12. Parameter estimates and bootstrap results for the final model

Parameter	Units	Parameter estimates	IIV (%) [Shrinkage (%)]	IOV (%) [Shrinkage (%)]	Bootstrap median (95%CI)		
					Parameter estimate	IVV %	IOV (%)
Clearance (CL/F)	L/h/5.6 kg	3.1	-	126.5 [23]	3.0 (2.9 – 3.5)	-	122.3 (101.1 – 147.4)
Volume of distribution (V _d /F)	L/5.6 kg	9.6	-	-	9.7 (9.1 – 11.7)	-	
Absorption rate constant (k _a)	/h	0.39	-	56.8 [16]	0.40 (0.28 – 0.48)	-	55.6 (26.5 – 75.8)
Reduction of relative bioavailability (F) for non-study days	-fold	3.2	69.5 [18]	-	3.2 (2.3 – 3.8)	70.8 (32.6 – 86.4)	-
Increase of F with increasing cholesterol above 3 mmol/L	%	20.7			12.8 (4.0 – 45.3)		
Proportional for <5 h	%	37.7	15.5 [20]	-	37.8 (30.7 – 44.8)	14.5 (5.7 – 22.6)	-
Proportional for >5 h	%	27.2		-	26.7 (18.8 – 35.6)		-

IOV: inter-occasion variability; IIV: inter-individual variability; CL/F: apparent clearance; V_d/F: apparent volume of distribution; CI: confidence interval

Final model for study days, given an F of 1:

$$CL \left(\frac{L}{h} \right) = 3.1 \times \left(\frac{FFM}{5.6} \right)^{0.75} \times (1 + 0.207 \times (CHOL - 3))$$

$$V_d(L) = 9.6 \times \left(\frac{FFM}{5.6} \right)$$

Inclusion of FFM, allometrically scaled, into the model, showing its influence on clearance (CL) and volume of distribution (V_d) improved the fit of the model ($\Delta\text{OFV} = -8.7$). Reduced adherence, based on the pre-dose sample taken on day 14, which is linked to the dose on the previous day, was identified as influential on F of LPV and reduced F 3.2 fold ($\Delta\text{OFV} = -19.3$). Further, increased cholesterol **was shown** to be linearly related to F, with a 20.7% increase in F for every 1 mmol/L increase in cholesterol above 3 mmol/L ($\Delta\text{OFV} = -4.8$). None of the other tested covariates including randomisation to early or delayed ART, tuberculosis co-medications nor anthropometrical measurements, explained the variability on CL, V_d or k_a .

The final LPV model was evaluated using a pred-varVPC and a bootstrap. Figure 1 demonstrates that the model describes the data well, particularly for day 1. However an under-prediction of the median peak concentrations on day 14 was noted, which could not be improved upon after intensive evaluation of differences between day 1 and day 14 and no further covariate inclusion. The bootstrap produced similar parameters estimates to the final model (Figure 9), indicating that the estimates for the population PK parameters in the final model are robust and stable.

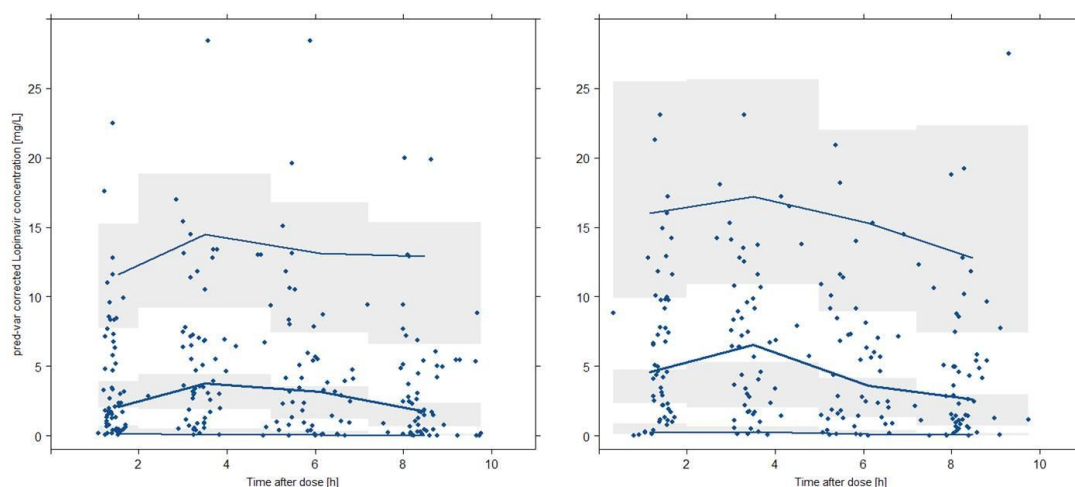


Figure 9. Lopinavir concentrations versus time after dose: Prediction- and variance- corrected visual predictive check for Lopinavir concentrations (mg/L) versus time after dose (h) for day 1 (left) and day14 (right). Blue dots represent concentrations observed in the children, blue lines represent the observed 2.5th, 50th, 97.5th percentile. The grey areas present the 90% CI around the model predicted 2.5th, 50th, 97.5th percentile.

Prediction- and variance- corrected visual predictive check for Lopinavir concentrations (mg/L) versus time after dose (h) for day 1 (left) and day14 (right). Blue dots represent concentrations observed in the children, blue lines represent the observed 2.5th, 50th, 97.5th percentile. The grey areas present the 90% CI around the model predicted 2.5th, 50th, 97.5th percentile

3.4.3 *Treatment Outcomes versus Pharmacokinetics*

Twenty-three patients had one or more of the nine samples which were measured below the lower LoQ, 13 of these patients had one sample below LoQ, five patients had two samples measured below LoQ, two patients had three samples below LoQ and three patients had four samples with concentrations below LoQ. Comparison of frequency of LoQ samples for patients with treatment failure to patients with treatment success showed no statistical difference at 12 weeks ($p=0.41$) and 48 weeks ($p=0.44$).

Comparison between patients with treatment failure to patients with treatment success of the individual estimates of apparent LPV clearance (CL/F) and LPV exposure ($AUC_{0-12\text{ h}}\cdot\text{mg/L}$) showed no statistical difference at 12 weeks ($p=0.89$, $p=0.95$) and 48 weeks ($p=0.92$, $p=0.65$), respectively. Results are displayed in Table 13.

Table 13. LPC concentrations below the Limit of Quantification (LoQ), individual apparent LPV clearance and LPV exposure (AUC₀₋₁₂) versus virological and treatment outcomes at week 12 and 48.

	Patients with concentrations below LoQ (%)	<i>p</i>-value*	CL (L/h) (median [IQR])	<i>p</i>-value*	AUC₀₋₁₂ (h·mg/L) (median [IQR])	<i>p</i>-value*
Treatment outcome week 12 (n= 54), LTFU=4, Missing VL=4						
Failure (n=38)	28%	0.41	4.5 [1.9 – 11.9]	0.89	23.6 [10.2 – 62.8]	0.95
Success (n=16)	42%		4.3 [2.1 – 12.6]		28.4 [9.0 – 61.8]	
Treatment outcome week 48 (n=58), LTFU =8, Missing VL=4						
Failure (n=17)	41%	0.44	3.2 [1.7 – 13.9]	0.92	25.5 [8.7 – 69.0]	0.65
Success (n=33)	30%		3.5 [2.0 – 10.4]		31.4 [11.8 – 60.8]	

*compared to 'Failure' category, n= number of patients included, LoQ- Limit of Quantification, LPV – lopinavir

3.5 Discussion

In this pharmacokinetic evaluation of LPV, severely malnourished children displayed significant pharmacokinetic variability, reduced bioavailability and consequently greater CL/F estimate of 0.6 L/h/kg in comparison to reports from other studies in non-malnourished children of LPV CL/F ranging from 0.2 to 0.4 L/h/kg (15,21,22). The findings are in keeping with the results from a recently published study conducted in Ugandan children, where there was a trend towards lower LPV bioavailability in malnourished compared to non-malnourished children. However in that study only 8% of the Ugandan study population was wasted and the evaluations were performed at least 14 days after ART initiation (23). In comparison, in this study, all patients were severely malnourished and exposures were measured on day 1 and 14 of ART initiation. We have found that observed peak concentrations were slightly increased on day 14 compared to those predicted by the model (see Figure 10), however the difference could not be explained by any of the available explanatory factors collected in the study. The increase could be due to recovery of the children in regards to their clinical condition as well as their nutritional status. LPV in serum is highly protein bound to albumin and alpha-1-acid glycoprotein (AAG), changes in AAG following nutritional rehabilitation, which was not measured in this study could account for this finding. A similar under-predicted peak concentration for LPV was noted in the study of Ugandan children (15).

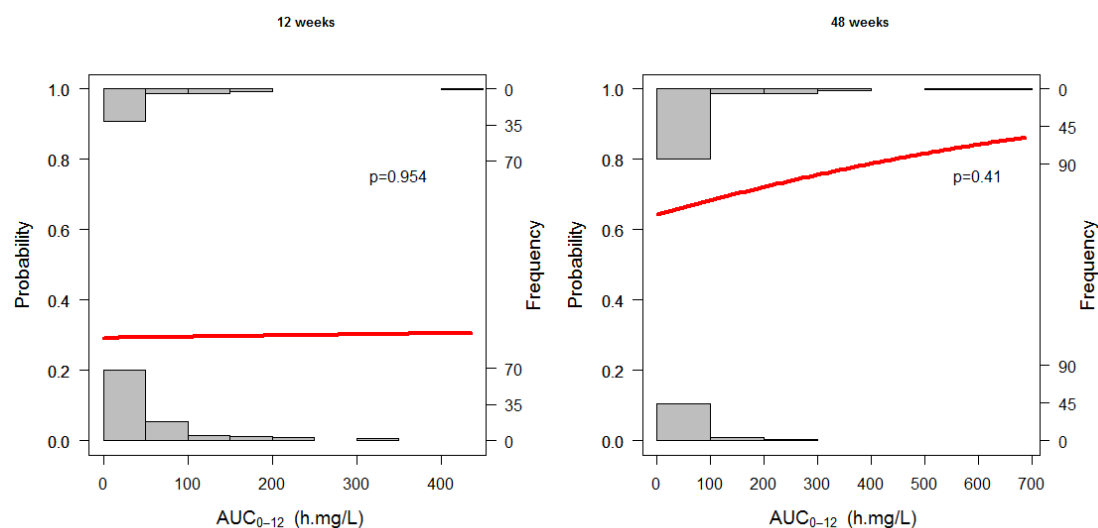


Figure 10. Distributions of LPV exposure (AUC_{0-12} , h.mg/L) for individuals with treatment success (top, probability = 1) and treatment failure (bottom, probability = 0) at week 12 (left) and 48 (right) post ART initiation. Treatment failure were defined as: death or viral load > 1000 copies/mL and treatment success as: alive and viral load < 1000 copies/mL. The red line presents the binary logist regression model illustrating the relationship between exposure and probability of treatment success. *P*-values presented in the graphic are greater than the significance level of 0.01, and therefore show that there is no statistically significant association between treatment success at week 12 and 48 and LPV exposure from day 1 and day14 of ART.

LPV/rvt based treatment has been demonstrated to result in superior virologic suppression rates in children less than 3 years of age (24,25), and therefore remains part of the WHO recommended first line regimen in young children (26). LPV pharmacokinetics has been well described in non-malnourished children and has formed the basis for the development of the WHO weight band dosages for LPV that were used in this study (27). As the majority of young children requiring ART reside in countries where up to 42% of children are malnourished at ART initiation (28), using standard mg/kg dosing may result in sub-optimal drug concentrations in a significant proportion of children initiated on ART. The role of LPV dose adjustment to achieve therapeutic dose during the acute phase of malnutrition needs to be further evaluated.

This study showed that FFM was superior to total body weight in describing variability around CL/F and Vd/F, which may be due to the relatively high FFM proportion of total weight. Initial weight gain in malnourished children especially if associated with stunting is predominately due to an increase in fat mass with lean body mass increasing later (29). The average time to ART initiation in the delayed arm was significantly longer (mean 6.2 vs. 23.7 days; $p=0.0001$). It is likely that a more prolonged delay in ART initiation is required for lean body weight to normalize. However delaying ART initiation comes at a potential risk of excess mortality and morbidity, (30) especially in children with advanced HIV disease. We failed to demonstrate that improved LPV exposure (AUC) during the acute phase of malnutrition predicted virologic failure or death at 12 and 48 weeks. However, sub-therapeutic plasma concentrations in children initiating ART early during nutritional recovery when the HIV viral load is high may result in archived mutations that can potentially affect long-term management. Dose adjustment of LPV may facilitate earlier ART initiation while achieving adequate LPV exposures **although the routine use of FFM to calculate doses in routine practice is impractical.**

Therapeutic drug monitoring (TDM) in hair or blood samples to detect sub-optimal drug concentrations has been used as a marker of virologic treatment failure and development of HIV drug resistance (31,32). In our study, neither the proportion of patients with LPV concentration below LoQ or estimates of apparent LPV clearance and LPV exposure (AUC) during the acute phase of malnutrition predicted virologic failure or death at 12 or 48 weeks. **However, sub-therapeutic plasma concentrations at ART initiation when the HIV viral load is high may result in archived mutations that can potentially affect long-term management.** In a paediatric study of LPV TDM from

samples collected between 10-80 weeks post ART initiation, sub-therapeutic LPV levels were linked with HIV drug resistance. The authors postulated that the sub-therapeutic LPV levels were most likely a surrogate marker of prolonged poor adherence resulting in the evolution HIV drug resistance (32). The sampling in our study was performed soon after ART initiation and sub-therapeutic plasma levels were most likely a result of altered pharmacokinetics of LPV associated with malnutrition, the effect of FFM and other factors such as inflammatory processes related to high burden of HIV and other opportunistic infections that might affect metabolic pathways. The altered pharmacokinetics due to these factors may reverse following nutritional recovery, returning the LPV plasma level to their normal while poor adherence may not, accounting for the lack of association in our study.

Mycobacterium Tuberculosis was a common diagnosis, requiring co-administration of “super-boosted” LPV/rtv (LPV/rtv with additional ritonavir to achieve a ratio of 1:1) in rifampicin co-treated patients in line with WHO and South African National ART Guidelines (33). In our study population of severely malnourished children, the approach using “super-boosted” LPV/rtv in children on tuberculosis treatment resulted in similar LPV exposures to children without tuberculosis (34). A population pharmacokinetic model developed by Zhang et al. suggested that the recommend doses of LPV/rtv needed to be increased in malnourished children with and without concomitant rifampicin-based tuberculosis treatment. The model predicted the doses of “super-boosted” LPV/rtv needed to maintain LPV trough concentrations > 1mg/L in 95% of children. Children in the 3-5.9 kg weight band needed close to twice the dose per kilogram of body weight (LPV/rtv 22/22 mg/kg) compared to the 14-19.9 kg weight band (LPV/rtv 12/12 mg/kg) (21). The weight band dosage table used in our study achieved similar doses (LPV/rtv 20/25mg/kg for children between 3-5.9 kg and 11.7/12 mg/kg for children between 14-19.9 kg) however still resulted in lower LPV exposures .

The estimated bioavailability of LPV was greatly reduced on days when no full pharmacokinetic sampling was undertaken (day 13, pre-dose), which is likely due to poor adherence or administration on these ‘non-observed’ days. All study patients remained in-patients between day 1 and day 14, the caregiver under the supervision of clinical staff administered ART. The poor palatability of LPV/rtv syrup due to the bitter taste often makes administration of this formulation difficult (35) and is the likely cause for this finding. Alternative formulations of LPV/rtv in young children have been

studied with variable success. Crushed LPV/rtv tablets do not result in adequate plasma levels (36,37), and while LPV/rtv “mini-pills” result in adequate plasma levels, the palatability of the formulation is still sub-optimal (38). A granule formulation with adequate taste masking is under development by Drugs for Neglected Diseases Initiative (DNDi) and in the future may result in a formulation with good palatability and tolerability (39).

Weaknesses: The inability to detect effects of TB co-treatment, and timing of ART initiation may be due to lack of power to do so given the extreme variability encountered in the data. Further the variable adherence of the in-patient cohort despite verification of administration in hospital prescription charts was not anticipated. The design therefore did not allow definitive accounting for the effect of adherence on LPV variability. Adherence could also have potentially confounded the evaluation of timing of ART initiation (delayed ART) and TB co-treatment.

3.6 Conclusions

The timing of ART initiation or the use of super-boosted LPV/rtv in TB co-infected patients did not affect LPV pharmacokinetics. Only FFM and cholesterol explained some variability in the pharmacokinetics of LPV in severely malnourished HIV-infected pediatric patients. As LPV pharmacokinetics was found to be highly variable and bioavailability greatly reduced, resulting in a greater CL/F estimate in comparison to other studies, doses of LPV/rtv may need to be adjusted in malnourished infants and young children initiating ART.

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BRIDGING TEXT:

In the literature review, the higher mortality associated with HIV-infected malnourished children compared to their uninfected counterparts was described. Although the mortality described in Chapter 2 was lower than that described in the literature for HIV-infected children with SAM, the mortality was higher than the WHO mortality norms for children with SAM. Majority of the mortality described occurred within the first 3 months following admission. The excess mortality is postulated to be related to bacterial infections on admission or acquired during hospitalization. In the following chapter the bacterial pathogens identified in this cohort of severely malnourished HIV-infected children is described and the relationship between the identification of bacterial pathogens and mortality is explored.

CHAPTER 4

BACTERIAL INFECTIONS IN HIV-INFECTED CHILDREN ADMITTED WITH SEVERE ACUTE MALNUTRITION IN DURBAN, SOUTH AFRICA

Bacterial infections in HIV-infected children admitted with severe acute malnutrition in Durban, South Africa

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Background: Bacterial infections in HIV-infected children admitted with severe acute malnutrition (SAM) contribute to higher mortality and poorer outcomes. This study describes the spectrum of bacterial infections in antiretroviral treatment (ART)-naïve, HIV-infected children admitted with SAM.

Methods: Between July 2012 and February 2015, 82 children were prospectively enrolled in the King Edward VIII Hospital, Durban. Specimens obtained on and during admission for microbiological evaluation, if clinically indicated, included blood, urine (obtained by catheterisation or suprapubic aspiration), induced sputum and cerebrospinal fluid. All positive bacterial cultures between admission and 30 days after enrollment were documented and characterised into samples taken either within 2 days of admission (infections on admission) or within 2–30 days of admission (hospital-acquired infections, HAIs).

Results: On admission, 67% of patients had abnormal white blood cell counts (WBC) (>12 or $<4 \times 10^9/L$) and 70% had elevated CRP; 65% were classified as severely immunosuppressed according to the WHO immunological classification.¹ A pathogen was isolated on the admission blood culture in four patients (6%) and in 27% of urine specimens. HAIs were predominately Gram-negative (39/43), and 39.5% were extended-spectrum β -lactamase-positive. Mortality was not significantly associated with isolation of a bacterial pathogen.

Conclusions: Routine pre-hospital administration of antibiotics as per the Integrated Management of Childhood Illness (IMCI) guidelines may be responsible for the low rates of positive admission blood cultures. HAIs with drug-resistant Gram-negative organisms are an area of concern and strategies to improve the prevention of HAIs in this vulnerable population are urgently needed.

Keywords: Bacterial Infections, Severe acute malnutrition, Paediatric HIV

Introduction

Effective prevention of mother-to-child transmission (PMTCT) has resulted in significant decreases in the incidence of new HIV infections in children, but, nevertheless, over 360,000 children <15 years of age are living with HIV in South Africa.² Furthermore, malnutrition remains a common paediatric diagnosis in developing countries. In 2008, the World Health Organization (WHO) estimated that 8.7% of children in KwaZulu-Natal (KZN) <5 years of age had weight-for-age (WAZ) scores 2SD or more from the median and 23.9% had height-for-age (HAZ) Z-scores 2 SD or more from the median.³ This is an improvement since 2005 when 12.8% of children had WAZ scores 3 SD

from the median. The South African Health and Nutrition Examination Survey (SANHANES) in 2012 reported a prevalence of 14% for stunting, 2.4% for wasting (weight for height $<2SD$ from the median) and 0.1% for severe wasting (weight for height $<3SD$ from the median) in KZN.⁴ Despite these improvements in the nutritional status of children, severe acute malnutrition (SAM) is estimated to be responsible for as much as 10% of all deaths in children aged <5 years globally.⁵

Malnutrition is a common clinical finding on initiation of antiretroviral treatment (ART) in sub-Saharan Africa and almost half of all children are malnourished.⁶ SAM in HIV-infected children is associated with poor clinical outcomes and high mortality.⁷ Bacterial infections such as bacteraemia or pneumonia are common and are associated with high mortality in children with SAM, but the

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clinical signs are often masked. Empirical antibiotic therapy significantly decreases the associated mortality and therefore is part of the package of care of children with SAM.⁸ HIV infection and its associated immunodeficiency also increase the risk and mortality of bacterial infections. Although HIV and SAM have not been shown to *further* increase the likelihood of bacterial infections, it may be associated with increased mortality.⁹

Although inpatient management is an essential component of the management of complicated SAM, it is recognised that hospital-acquired infections (HAIs) are an increasing problem across sub-Saharan Africa, including in paediatric wards.¹⁰ Antibiotic resistance no longer exists only in well resourced countries, and indeed colonisation with resistant Gram-negative organisms has been demonstrated to spread rapidly throughout paediatric nutrition stabilisation centres.¹¹ Therefore, it is imperative that empirical antibiotic therapy for suspected HAIs in malnourished children be sufficiently broad spectrum to cover likely pathogens, but equally this must be backed up by adequate diagnostic microbiology facilities to ensure the identification and sensitivity testing of the responsible organism, thus aiding the switch to targeted therapy. However, such facilities are not always available in settings where SAM is common.

King Edward VIII Hospital (KEH) in the eThekweni District of KwaZulu-Natal, has a 100-bed paediatric ward (including ten nutritional rehabilitation beds) which provides regional and tertiary-level care. A randomised controlled trial in children presenting with SAM and HIV was undertaken to assess the clinical and pharmacokinetic responses to early versus delayed antiretroviral therapy.¹² Eligible children were randomised either to commence ART within 14 days of admission (early arm) or following nutritional recovery, and to commence ART >14 days after admission (delayed arm). All patients received the same standardised nutritional intervention according to the WHO guidelines for the management of SAM in children with standardised re-feeding guidelines.¹³

All bacterial pathogens isolated from this unique cohort of vulnerable children within the first 30 days of admission are described.

Methods

All children presenting with SAM (weight-for-length Z-score <3 SD from the median or mid-upper-arm circumference <11.5 cm and/or oedema) who were newly diagnosed with HIV infection were eligible for inclusion in the Malnutrition and Antiretroviral Timing in Children with HIV (MATCH) trial.¹³ The hospital protocol recommended that blood cultures be performed on admission for all children with SAM. In cases of pyrexia or unexplained deterioration, urine, sputum, cerebrospinal fluid (CSF) and additional blood cultures were undertaken during admission at the discretion of the clinician.

Laboratory methods

Blood cultures were obtained using standard aseptic techniques following cleansing of the skin with 2% chlorhexidine/alcohol solution. The recommended blood volume inoculated into blood culture bottles was 1–3 mls, as per the manufacturer's recommendations. Induced sputum samples were obtained with the assistance of a physiotherapist, and urine samples were obtained either by in-out catheterisation or suprapubic aspirate. Significant bacteriuria was defined as >10⁵ colony-forming units (CFU)/ml of urine. This was determined by quantitative culture of the urine sample using a calibrated loop.

The laboratory uses the BacT/ALERT® (BioMérieux, France) fully automated, continuous monitoring blood culture system. When a blood culture bottle is detected as positive, Gram staining is performed on the broth for presumptive identification. The blood culture medium is then subcultured onto agar plates to obtain bacteria colonies which are identified using biochemical testing. The Vitek II (BioMérieux, France) automated system is used for identification and sensitivity testing, as recommended by the Clinical Laboratory Standards Institute. Detection of extended-spectrum β-lactamases (ESBL) is based on comparison of the inhibitory effects of ceftazidime, cefotaxime and cefepime, alone and in combination with clavulanate.¹⁴

SAM was managed according to the WHO Guidelines for the Management of Severe Acute Malnutrition.¹³ All positive bacterial cultures associated with each patient between admission to hospital and 30 days after enrolment in the study were recorded and characterised into one of two groups, whether the samples were taken within 2 days of admission (indicating infection on admission) or 2–30 day after admission (indicating HAIs). The clinical and laboratory parameters of patients with positive cultures were evaluated by a clinical microbiologist and a specialist in paediatric infectious diseases to determine whether the isolated organism was a pathogen requiring an anti-microbial agent or a possible contaminant. The full antibiotic sensitivity profile for each organism isolated was recorded. The South African National Health Laboratory Service's online record system was used to identify positive cultures taken when children were initially admitted with SAM to another institution before transfer to KEH and enrolment in the study.

Routine laboratory results were accessed from the day of admission via the online laboratory record system. CD4 counts and viral loads obtained within 2 weeks of admission were also recorded. Our local protocol dictates benzylpenicillin and gentamicin as first-line empirical antibiotics for severely unwell children. Piperacillin–tazobactam and amikacin are second-line, and carbapenems third-line (meropenem). Imipenem replaced meropenem during the latter phases of the study. Quinolones are prescribed only if indicated by culture results.

Statistical analysis

The available baseline demographic and laboratory parameters in all patients with a positive culture within 2 days of admission were compared with those taken 2–30 days after admission. A descriptive analysis of all pathogens isolated within 30 days of each patient's admission to hospital is presented. If a sample grew multiple pathogens, that patient was analysed as though they had a single positive culture, but all organisms were recorded for the purposes of describing the bacteriological spectrum.

Variables explored in univariate analysis included age, sex, CD4 count and percentage, white cell count (WCC), haemoglobin (Hb), platelet count, albumin and C-reactive protein (CRP) levels. The means of the above variables were compared between the two groups using the unpaired *t*-test. In addition, these variables were dichotomised and categorised on the basis of the parameters which would normally be expected to indicate severe infection – WCC >12 or <4 × 10⁹/L¹⁵, Hb <7 g/dL¹⁶, platelets <100 × 10⁹/L, albumin <25 g/L, CRP >10 mg/L, and severe immunosuppression as classified by the WHO [CD4% <25% (age <12 months), <20% (age 12–35 months), <15% (age 36–59 months), and CD4 count <200 cells/μL or <15% (age ≤5 years)].¹⁷ Percentages among these categorical variables were compared using the χ^2 or Fischer's Exact test, as appropriate. Thirty-day survival between all patients with positive cultures and those with negative cultures were compared using the log rank test. Statistical analyses were performed using SPSS version 20 (IBM). No imputations were made for missing variables. All tests were two-tailed and a *P*-value of <0.05 was considered to be significant.

Ethics approval and funding

Written informed consent was obtained from the caregivers of all children enrolled in the MATCH study. The trial was approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal and King Edward VIII Hospital.

Results

Baseline characteristics

A total of 82 children were enrolled in the MATCH study. Mean age on admission was 23 months (range 1 month to 10.6 years) and 44 (53.6%) were boys. Eighteen children (21.95%) presented with oedematous malnutrition. A full blood count and basic biochemical profile was undertaken for 81 on the day of admission and a CD4 count was performed in 73 within 2 weeks of admission.

The mean WCC on admission was 13.95 × 10⁹/L; in 55 children (67.1%), it was either >12 or <4 × 10⁹/L on admission. Mean haemoglobin on admission was 8.79 g/dL, and in 14 children (17.1%) Hb was <7 g/dL on admission. The mean platelet count was 330 × 10⁹/L, and ten children had platelet counts <100 × 10⁹/L. The mean albumin level was 23.47 g/L; 44 patients (53.7%) had albumin levels <25 g/L

on admission. CRP levels on admission were measured in 37 children, and the mean CRP was 40.5 mg/L, with 26 children (70%) having CRP levels >10 mg/L (normal 0–5).

CD4 counts on admission ranged from 2 to 3354 cells/μL and the mean CD4 cell percentage on admission was 17.4%; 53 (64.6%) children were classified as severely immunosuppressed according to WHO criteria. In addition to standard bacteriological investigations and treatment, 21 children had proven or suspected tuberculosis; these children will be described in a separate report.

Culture samples

Blood cultures were performed on the day of admission in 71 children, four of which were positive for pathogens (three *Staphylococcus aureus*, one non-typhoidal *Salmonella*) and 32 were reported as contaminated, mainly by Gram-positive organisms (*Bacillus* and *Staphylococcus* species), but also one grew mixed Gram-negatives, including *Acinetobacter* with no clinical or biochemical evidence of infection. Blood cultures were performed in 46 children at some point during their hospital stay, at a mean of 11 days into their admission; 14 of these grew contaminants and 11 were positive for pathogens.

Urine cultures were performed in 53 children: 41 on admission, 11 of which were positive, and 12 taken within 2–30 days of admission, six of which were positive. Rates of bacteriuria were similar in girls (21%, *n*=8) and boys (20.9%, *n*=9). Urine microscopy demonstrated leucocytes in 90% of patients with a positive culture.

Sputum culture was undertaken in 38 children, three on the day of admission, one of which was positive for bacteria, and 35 within 2–30 days of admission, 14 of which were positive. All patients with a positive culture had leucocytes present on sputum microscopy.

Lumbar puncture was performed on 19 children, and all CSF cultures were negative; one sample had an elevated lymphocyte count and protein, and the child was treated empirically for tuberculous meningitis.

Thirty-nine children had at least one positive culture sample. One child had both admission- and hospital-acquired bacteraemias (*S. aureus* and *Acinetobacter baumannii*, respectively) plus a positive sputum culture (*Klebsiella pneumoniae*). Five children had positive cultures from two sites: one had positive hospital-acquired blood and sputum cultures (*S. aureus* in sputum, *Escherichia coli* in blood); one had positive urine and sputum cultures (*K. pneumoniae* in urine, *E. coli* in sputum) and three had positive urine cultures and hospital-acquired blood cultures (the same organism, *K. pneumoniae*, grew from both in two cases, with an identical resistance pattern in one instance, while different organisms grew in each sample in the third case (*E. coli* in urine, *Enterobacter cloacae* in blood).

Three children grew multiple organisms from a single sample: one had two organisms (*Streptococcus pneumoniae* and *S. aureus*) and one had three organisms (*K. pneumoniae*, *E. coli* and *S. aureus*) in sputum, while

Table 14. Admission cultures: comparison of 82 patients with positive vs negative cultures

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Table 1 Admission cultures: comparison of 82 patients with positive vs negative cultures

	Culture negative in first 2 days, n = 66	Any positive sample within 2 days of admission, n = 16	P-value
Mean age, mths (SD)	23.6 (29.4)	20.1 (23.3)	0.73
Male (%)	36 (54.5)	8 (50)	0.74
Oedema on admission (%)	13 (19.7)	5 (31.2)	0.33
CD4 count, cells/ μ L (SD)	933 (826)	758 (687)	0.42
CD4 cell % (SD)	17.37 (9.8)	17.83 (8.8)	0.87
Severe immunosuppression (%)	44 (74.6)	9 (64.3)	0.43
TB (%)	18 (27.3)	3 (18.8)	0.75
Early ART, <2 wks (%)	33 (50.0)	5 (31.2)	0.26
White cell count $\times 10^9$ /L (%)	13.83 (6.64)	14.46 (7.9)	0.75
WCC >12 or <4 $\times 10^9$ /L (%)	43 (65.2)	12 (75.0)	0.45
Haemoglobin, g/dL (%)	8.75 (2.14)	8.94 (2.1)	0.76
Haemoglobin, <7 g/dL (%)	11 (16.7)	3 (18.8)	1.0
Platelet count $\times 10^9$ /L (SD)	327 (201)	342 (180)	0.8
Platelets <100 $\times 10^9$ /L (%)	9 (13.6)	1 (6.7)	0.68
CRP, mg/L (SD)	40 (37.0)	42 (61.0)	0.91
CRP >10 mg/L (%)	19 (76)	7 (58.3)	0.27
Albumin, g/L (SD)	23 (7.0)	24 (9.0)	0.75
Albumin <25 g/L (%)	36 (55.4)	8 (50.0)	0.7

Severe immunosuppression is based on the WHO 2006 classification.¹ TB includes both bacteriologically confirmed and clinically diagnosed cases. ART, antiretroviral therapy; CRP, C-reactive protein; normal values: CRP 0–5 mg/L and albumin 28–48 g/L.

one had two hospital-acquired organisms (*K. pneumoniae* and *Serratia marcescens*) in a blood culture.

Table 1 compares the 16 children who had positive bacterial cultures (4 blood cultures, 11 urines, 1 sputum) within 2 days of admission with those whose cultures were taken within 2 days and were negative. No statistically significant differences in baseline characteristics were found between the two groups.

Survival

Two patients died within 30 days of admission; one had a positive urine culture (*E. coli*) on admission and the other had hospital-acquired bacteraemia (*S. marcescens*) on day 15. The difference in 30-day mortality between patients with identifiable pathogens (5.1%) and those who remained culture-negative was not statistically significant.

Descriptive analysis of bacterial isolates

A total of 43 Gram-negative and 8 Gram-positive pathogens were isolated from 39 patients. Table 2 summarises all Gram-negative isolates and Table 3 the Gram-positives. *Klebsiella* (predominantly *K. pneumoniae*) was the commonest bacterial isolate from any and all sites ($n=17$), followed by *E. coli* (11), *Staphylococcus aureus* (6), *Acinetobacter* species (4) and *Pseudomonas aeruginosa* (3). *Acinetobacter* was the second most common nosocomial blood culture isolate, while *E. coli* was the second most common urinary pathogen. Only one non-typhoidal *Salmonella* species was isolated. Generally speaking, Gram-positive organisms tended to be susceptible to most first-line antibiotics, and resistance was far more prevalent among Gram-negative pathogens. Extended-spectrum β -lactamases were identified in 39.5% (17), including 13 of 19 *Klebsiella* isolates. There was ciprofloxacin resistance in 12 isolates across a range of species. Only two Gram-negatives were susceptible to amoxicillin (11 to

amoxicillin–clavulanate) and two others were susceptible to trimethoprim–sulfamethoxazole.

Carbapenems and amikacin appeared to be the most active agents against Gram-negative organisms. Sensitivities to imipenem, ertapenem and colistin only began to be analysed in late 2013, and resistance to these agents was low; the Vitek II (BioMérieux, France) automated system which was used tests the organisms against a panel of antibiotics, irrespective of their inherent resistance mechanisms.

Organisms such as the *Serratia* spp. and the *Morganella* spp. isolated in two blood cultures and one urine are known to be naturally resistant to colistin.

All organisms, except for a single *P. aeruginosa* isolate, were susceptible to meropenem and imipenem and no ertapenem resistance was detected (although only 17 isolates were tested).

Discussion

In a prospective cohort of 82 South African children with HIV infection admitted with SAM to an urban teaching hospital, 47.5% were culture-positive for pathogenic bacteria at least once during the first 30 days of their admission. Gram-negative pathogens were isolated from a variety of sites in 33 patients, including 11 episodes of hospital-acquired bacteraemia. Seventeen of 43 Gram-negative isolates were ESBL producers. Univariate analysis did not detect any predictors of positive culture samples on admission in children with SAM and HIV, and the trend towards increased 30-day mortality in children with positive bacterial cultures did not achieve statistical significance; however, the study was not powered to detect mortality differences.

Bacteraemia is common in children with HIV and/or SAM; 17% of the children had at least one episode of

Table 15. Hospital-acquired infections: description of Gram-negative organisms cultures

Table 2 Hospital-acquired infections: description of Gram-negative organisms cultured

	All Gram-neg- atives, n = 43	Klebsiella species*, n = 19	Escherichia coli, n = 11	Acin etobacter species†, n = 4	Pseudomonas aeruginosa, n = 3	Serratia mar- ascens, n = 2	Salmonella species, n = 1	Enterobacter cloacae, n = 1	Morganella morganii, n = 1	Haemophilus influenzae, n = 1
Blood	13	4	1	3	1	2	1	1		
Urine	16	8	7	1	2	0	0	0	1	1
Sputum	14	7	3	0	2	0	0	0	0	0
ESBL producers	17	13	2	0	2	0	0	0	0	0
Sensitivities:										
Amoxicillin	2	0	0	0	0	0	1	0	0	1
Co-amoxiclav	11	2	7	0	0	0	1	0	0	1
Piperacillin- tazobactam	26	7	10	3	1	2	1	0	1	1
Cefuroxime	11	2	8	0	0	0	0	0	0	1
Cefixitin	30	17	10	0	0	1	0	0	1	1
Ceftazidime	17	2	8	3	1	2	0	0	0	1
Ceftriaxone	14	2	8	0	0	2	1	0	0	1
Ciprofloxacin	30	12	9	3	1	2	1	1	1	1
Gentamicin	20	5	9	1	1	2	1	0	1	1
Amikacin	33	13	10	4	2	2	1	1	1	1
Meropenem	42	19	11	4	2	2	1	1	1	1
Imipenem	18	10‡	4‡	2‡	1	2	1	1	1	1
Ertapenem	15	9‡	6‡	3‡	0	0	0	1	0	0
Colistin	20	5‡	9‡	0	0	1	0	0	0	0
TMP-SMX	2	1	0	0	0	1	0	0	0	0

Intermediate-susceptible organisms were considered non-susceptible for purposes of this table. If a particular agent was simply not tested against a particular isolate, that isolate could not contribute to the number of organisms susceptible to that agent. ESBL, extended-spectrum β-lactamase.

*One *K. oxytoca* grew in a urine culture, all others were *K. pneumoniae*.

†All but one were *A. baumannii*; one (in a blood culture) was not identified further.

‡Not all isolates were tested, but all tested isolates were susceptible

Table 16. Description of all Gram-positive organisms cultured

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Table 3 Description of all Gram-positive organisms cultured

	All Gram-positives, n=8	<i>S. aureus</i> , n=6	<i>S. pneumoniae</i> , n=1	<i>Enterococcus faecium</i> , n=1
Blood	3	3*		
Urine	1			1
Sputum	4	3	1	
Amoxicillin*	2	0	1	1
Co-amoxiclav*	4	2	1	1
Flucloxacillin*	6	6		
Erythromycin*	6	5†	1	
Clindamycin*	6	5†	1	
Gentamycin*			1	
Vancormycin*	8	6		1

*Antibiotic susceptibility; †All from blood cultures taken on admission.

bacteraemia in the month following admission with SAM. A large study in Kenya between 1998 and 2002 found that 6.6% of all acute paediatric admissions had bacteraemia, and that HIV and malnutrition were independently associated with bacteraemia;¹⁸ studies exclusively of severely malnourished children have found bacteraemia rates of 12–22%.^{19–21} Empirical antibiotic therapy reduces mortality in all children admitted with SAM,⁸ but malnourished children with proven bacteraemia still have higher mortality,²⁰ and mortality from bacteraemia is higher in HIV-infected malnourished children than in those who are malnourished but not infected by HIV.¹⁹

Only four patients had culture-proven bacteraemia on admission, but prior antibiotic administration may have resulted in a falsely low detection rate. This is indirectly supported by the biochemical evidence of a significant acute inflammatory response in the majority of patients, suggesting the presence of bacteraemia despite being culture-negative. All the patients would be classified as 'very severe disease' by the South African Integrated Management of Childhood Illnesses (IMCI) Guidelines and were given a stat dose of ceftriaxone at a primary care facility before transfer to hospital. Therefore, prior antibiotic administration, even stat pre-transfer antibiotics, may decrease the yield from admission blood cultures. However, this needs to be weighed against the risks associated with delayed antibiotic administration in very sick children, especially where there are delays in transfer of patients.

S. aureus was responsible for three of the four positive admission blood cultures; however, our empirical antibiotic regimens for children with SAM have poor anti-staphylococcal coverage. Staphylococcal bacteraemia is common in South African children with HIV, regardless of nutritional status.²² While all of the *S. aureus* isolates in the population were fully susceptible, studies in other provinces have shown high rates of community-acquired methicillin-resistant *S. aureus* (MRSA) colonisation and bacteraemia in South African children with HIV.^{22,23} Prophylactic TMP-SMX has been associated with MRSA carriage,²³ but the majority of patients were newly diagnosed with HIV at the same time as their presentation with SAM, and thus were not prescribed regular TMP-SMX

pre-admission. High rates of non-typhoidal *Salmonella* bacteraemia were not observed in the cohort, contrary to the high rates seen throughout the rest of sub-Saharan Africa.^{17,18,22} This could be explained either by specific population demographics of the region, or by aggressive antibiotic therapy in the community and in hospital prior to blood cultures being taken, masking the rate of true positive cultures.

The higher rate of hospital-acquired than community-acquired bacteraemia in the HIV-infected malnourished children, and the high rates of antibiotic resistance in these pathogens is of concern. A study in Kenya found that hospital-acquired bacteraemia was more frequent and associated with a higher mortality than community-acquired bacteraemia (relative incidence 40:1, case fatality 53% vs 24%).²⁴ The Kenyan study differed in that all paediatric admissions were included, and HIV status was known in only 30% of patients. However, a similar spectrum of organisms was found, and poor nutritional status was also an independent risk factor for hospital-acquired bacteraemia. Infection with enterobacteriaceae, particularly resistant organisms, has been found to be strongly predictive of mortality in African children.²⁵ ESBL-producing enterobacteriaceae are an increasingly important problem throughout sub-Saharan Africa, and high rates of carriage have been shown in a variety of settings, including nasopharyngeal swabs of non-hospitalised HIV-infected children in Cape Town²⁵ and faecal samples of children admitted to a Médecins sans Frontières re-nutrition centre in Niger;¹¹ this latter study also showed the rapid spread of such organisms throughout the ward. Translocation of enteric bacteria into the circulation has been demonstrated in well nourished children with HIV, and shown to continue even after viral suppression with ART;²⁶ the rate of translocation in severely malnourished children with HIV may well be higher, and this may partially explain the high rates of Gram-negative bacteraemia in these children.

Urine was the commonest specimen from which a pathogen was isolated, with significant bacteriuria in 32% of cultures. There are few good quality studies of urinary tract infections in malnourished children in the HIV era, but older studies have found significantly increased rates of bacteriuria in malnourished children (13–15%).^{27,28}

A prospective study in The Gambia in 2011 found a 16.5% rate of bacteriuria in severely malnourished children, 20.5% of whom were newly diagnosed with HIV.²⁹ Another recent study found that a urine dipstick positive for nitrites or leucocyte esterase was strongly associated with mortality in SAM, although a positive urine culture was not found to predict death in this same study.³⁰ Rates of bacteriuria were equal in girls and boys which is consistent with most previous studies of malnourished children,^{27,28} although dipsticks were more often positive in girls.³⁰

Sputum was also a significant source of pathogens in this study. While not all sputum isolates necessarily represent invasive bacterial syndromes, the presence of these pathogens is of concern, given that the children in question were immunocompromised on several levels, both from HIV, immune paresis in the setting of severe malnutrition and compromised skin and mucosal integrity, and colonisation may result in invasive disease. Other authors have identified a similar spectrum of pathogens in sputum samples from HIV-infected children to that seen in this study.^{31,32} The diagnosis and management of pneumonia in malnourished children is not straightforward,^{29,33} and the exact role of sputum culture for bacteria in children with SAM (with or without HIV) is an important area for future research.

Limitations of the study include a single-centre setting and a relatively small cohort. Although the patients were referred from many facilities in KwaZulu-Natal, they might not be representative of children in other regions of South Africa or other sub-Saharan countries. However, the data were collected in the course of a prospective clinical trial that was both comprehensive and relatively bias-free compared with the inherent disadvantages of retrospective studies or cohort studies in other sites where universal HIV testing was not achieved. The study also had access to a fully equipped diagnostic microbiology laboratory which allowed for comprehensive microbiological evaluation.

Increasing rates of drug-resistant HAIs pose a serious challenge in the management of HIV-infected children with SAM. This study will prove useful to all clinicians caring for malnourished HIV-infected children in sub-Saharan Africa, and to those responsible for drawing up guidelines for their management. Strategies to reduce hospitalisation or length of stay in HIV-infected children with SAM would be expected to reduce the risk of nosocomial bacteraemia and improve outcome. Escalation of antibiotic coverage and spectrum in the face of clinical deterioration is clearly necessary for individual patients, but is not sustainable at the population level. Therefore, there needs to be increased investment in antibiotic stewardship programmes, hygiene initiatives and isolation facilities in centres caring for these patients, and the need to expand medical, social and financial interventions to tackle HIV and malnutrition in South Africa and beyond should be highlighted.

Conflict of Interest

None.

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BRIDGING TEXT:

South Africa is one of the 30 tuberculosis (TB) high burden countries, which collectively account for 85-89% of the global burden of TB disease. In addition to bacterial infections, we postulate that the excessive mortality described in the literature review and chapter 2 in severely malnourished HIV-infected children is due to a higher burden of TB disease. Childhood, malnutrition and HIV infection can independently increase the risk of TB disease, however few studies have described the cumulative risk of all three factors. In the following chapter we describe the frequency of both clinically diagnosed and culture confirmed cases of TB in severely malnourished children.

CHAPTER 5

TUBERCULOSIS IN HIV-INFECTED SOUTH AFRICAN CHILDREN WITH COMPLICATED SEVERE ACUTE MALNUTRITION

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Contributions:

All authors had a role in the design of this study. H Adler, M Archary and P Mahabeer collected the data. H Adler performed statistical analyses and wrote the first draft of the paper, which was revised with contributions from all authors. All authors have approved the final version

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5.1 Summary

Setting: Academic tertiary referral hospital in Durban, South Africa

Objective: To describe the incidence and diagnostic challenges of TB in HIV-infected children with severe acute malnutrition (SAM)

Design: Post-hoc analysis of a randomised controlled trial that enrolled ART-naïve, HIV-infected children with SAM. Trial records and hospital laboratory results were explored for clinical diagnoses and bacteriologically confirmed cases of TB. Negative binomial regression was used to explore associations with confirmed cases of TB, excluding cases where the clinical diagnosis was not supported by microbiological confirmation.

Results: Of 82 children enrolled in the study, 21 (25.6%) were diagnosed with TB, with bacteriological confirmation in 8 cases. Sputum sampling (as opposed to gastric washings) was associated with an increased risk of subsequent diagnosis of TB (adjusted relative risk 1.134, 95% CI 2.1%—26%). A culture-proven bacterial infection during the admission was associated with a reduced risk of TB (aRR 0.856, 0.748—0.979), which may reflect false negative microbiologic tests secondary to empiric broad spectrum antibiotics.

Conclusion: TB is common in HIV-infected children with SAM. While microbiological confirmation of the diagnosis is feasible, empiric treatment remains common, possibly

influenced by suboptimal testing and false negative TB diagnostics. TB investigation should be integrated into the programmatic management of HIV and SAM.

5.2 Introduction

Tuberculosis (TB), HIV infection and severe acute malnutrition (SAM) are individually responsible for high levels of morbidity and mortality among children across sub-Saharan Africa. Research is ongoing into how these three interact, although the potency of the combination has been recognised for some time (1).

Over 650,000 cases of paediatric TB occur annually in the 22 highest-burden countries (2), resulting in an estimated 140,000 annual deaths (3). Over 2.6 million children across the world are living with HIV, with 90% of these living in sub-Saharan Africa (4). HIV was responsible for 150,000 childhood deaths in 2014. SAM is responsible for as much as 10% of all global mortality in children aged <5 years (5), with severe wasting alone accounting for over 500,000 deaths annually in this age group (6).

In South Africa, over 36,000 cases of childhood TB occur annually, one quarter of which are believed to be HIV co-infected (7,8). HIV is responsible for 17% of all deaths in South African children aged under 5 years (8). One study found that 10% of children initiating antiretroviral therapy (ART) in rural South Africa also had SAM (9).

While SAM combined with HIV has already been recognised as a challenging clinical entity, associated with increased mortality (10,11), the contribution of TB in this scenario is uncertain. Malnutrition is associated with increased mortality in paediatric TB (12), particularly with HIV co-infection (13). TB causes cachexia and wasting, and may itself be impacted by poor nutritional status (14,15). HIV is an independent risk factor for both TB and malnutrition, worsening the outcomes of either condition (10,16). A description of TB in a cohort of paediatric patients who all have the specific combination of both HIV and SAM has not yet been studied. A randomised controlled trial (Malnutrition and Antiretroviral Timing in Children with HIV, MATCH) assessing the clinical and pharmacokinetic responses to early versus delayed ART in HIV-infected children with SAM was conducted in Durban, South Africa (17). We present a post-hoc analysis of TB diagnoses in these patients.

5.3 Study Population and Methods

5.3.1 Setting

King Edward VIII Hospital (KEH) has a 100-bed paediatric ward, including 10 nutritional rehabilitation beds, providing regional tertiary-level care.

5.3.2 Recruitment and Eligibility

All children presenting with SAM and newly diagnosed with HIV were eligible for inclusion in the trial, and randomised either to early (within ten days of admission) or delayed

(at four weeks or at protocol-defined nutritional recovery) ART (17). Recruitment was carried out between 2012 and 2014.

SAM was defined as weight-for-height z score below -3 standard deviations from the World Health Organisation (WHO) mean or a mid-upper-arm circumference of <115mm, with or without bilateral oedema (18) and was managed according to the latest WHO guidelines (19).

Patients were classified as bacteriologically confirmed TB (based on positive sputum smear microscopy, mycobacterial culture or nucleic acid amplification), clinically diagnosed (empirically treated) TB, or no TB, as per the WHO (20). A more detailed classification of clinically-diagnosed cases was not possible, as patients' original files and radiographs were frequently unavailable.

5.3.3 Data Collection

We retrospectively searched the National Health Laboratory Service's laboratory information management system for TB diagnostic test results from all patients in the MATCH trial, from the date of admission with SAM to any hospital in Durban, through to 1 month after transfer to KEH and enrolment in the study. We also recorded routine biochemical, haematological and immunological laboratory results from the day of admission. Clinical parameters and tuberculin skin test results at admission were not captured routinely, as children were only enrolled after admission and a positive HIV test.

Investigations for pulmonary TB were performed on at least three samples of induced sputum or gastric washings, at the discretion of the treating clinician. Fluorescent microscopy was performed on sputum smears, followed by culture in mycobacterial growth indicator tubes. Specimens that were smear positive and cultures that flagged positive were further subjected to polymerase chain reaction by line-probe assay (LPA; MTBDRplus, Hain Lifescience, Germany). In-vitro drug sensitivity testing was performed for both first and second line anti-tuberculosis drugs. The Xpert MTB-RIF platform (hereafter referred to as Xpert) was introduced in late 2013, replacing sputum smear microscopy.

5.3.4 Statistical Analysis

We present a descriptive analysis of all TB diagnoses in the MATCH study. TB-negative patients, bacteriologically-confirmed TB cases and clinically-diagnosed cases were compared using ANOVA (for continuously-distributed data) or chi-square (for categorical variables). In addition, we assessed associations between patient characteristics (including laboratory results, TB sampling method and ART strategy) and the diagnosis of TB using a negative binomial logistic regression model comparing children with and without a confirmed diagnosis of TB; empirically-treated children were excluded from this model, as were any children with missing data. We hypothesised that anaemia (Hb <10g/dL), leukocytosis (white cell count >12 x 10⁹),

thrombocytosis (platelets $>400 \times 10^9$) and hypoalbuminaemia ($<25\text{g/L}$) might, as surrogate markers of inflammation, be associated with a diagnosis of TB (ESR and CRP levels were infrequently measured, and therefore were not included in the model). Following univariate analysis, all variables were included in a fully-adjusted multivariate model. A parsimonious adjusted model was then reached via a backward stepwise approach, sequentially excluding variables whose p value was >0.1 and selecting a final model that minimised the Akaike information criterion. Results are presented as adjusted relative risks (aRR) with 95% confidence intervals (CI), and p values <0.05 were considered significant. All analyses were performed using SPSS version 22 (IBM).

5.3.5 Ethics

Written informed consent was obtained from the caregivers of all children enrolled in the MATCH study. The trial was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal and King Edward VIII Hospital.

5.4 Results

5.4.1 Descriptive Analysis

We enrolled 82 children in the MATCH trial. Figure 11 outlines the recruitment and TB diagnostic process, while Table 17 outlines the patients' baseline characteristics and sites of TB. The mean age at admission was 23 months, 46.3% ($n = 38$) were girls, the mean CD4 percentage was 17.46% and 21.95% ($n = 18$) presented with oedematous malnutrition. **The mean duration between initiation of TB treatment and ART was 16.4 days (SD 11.6).**

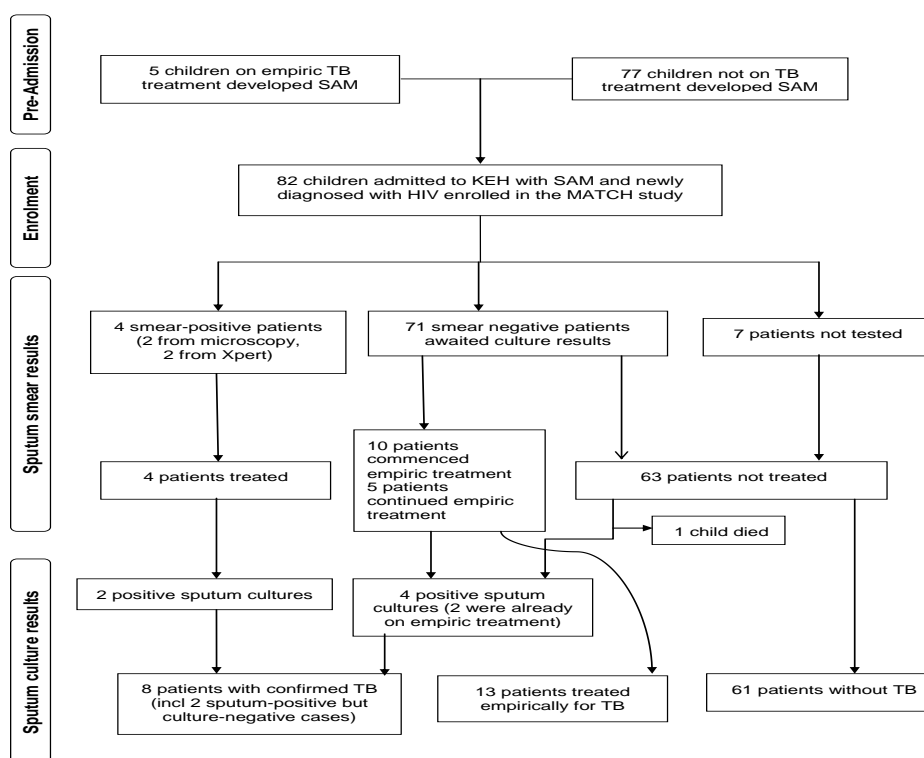


Figure 11. Recruitment and diagnostic process

Table 17. Baseline characteristics of the patients in this study

	TB negative (n = 61)	Bacteriologically confirmed (n = 8)	Clinically diagnosed (n = 13)	p
BASELINE CHARACTERISTICS				
Age (months)	19 ± 22	29 ± 41	36 ± 40	0.1
Female	28 (45.9%)	5 (62.5%)	5 (38.5%)	0.56
Oedema present	12 (19.7%)	4 (50%)	2 (15.4%)	0.12
Early ART (<2 weeks)	29 (47.5%)	3 (37.5%)	6 (46.2%)	0.87
Other bacterial infection	33 (54.1%)	3 (37.5%)	3 (23.1%)	0.1
CD4 (cells/μL) (n = 73)	945 ± 807	874 ± 309	721 ± 214	0.49
CD4 percentage (n = 73)	18.35 ± 9.1%	15.5 ± 9.83%	14.5 ± 11.4%	0.4
Haemoglobin (g/dL)	8.65 ± 2.0	8.7 ± 3.8	8.4 ± 1.7	0.73
Haemoglobin <7	9 (14.8%)	3 (37.5%)	2 (15.4%)	0.27
Platelets (x 10 ⁹ /L)	336 ± 208	336 ± 215	297 ± 131	0.81
Platelets >400	20 (32.8%)	1 (12.5%)	4 (30.8%)	0.6
Albumin (g/L)	24 ± 8	21 ± 5	23 ± 6	0.56
Albumin <25	30 (29.2%)	6 (75%)	8 (61.5%)	0.35
CRP (mg/L) (n = 37)	38 ± 49	38 ± 41	57 ± 35	0.68
CRP >10 (n = 37)	16	5	5	0.17
SITE OF TB				
Pulmonary	-	7	8	-
Pulmonary + Disseminated	-	1	3	-
Meningitis	-	0	1	-
Abdominal	-	0	1	-
DIAGNOSIS				
Smear microscopy	-	2*	-	-
Xpert	-	2†	-	-
Culture	-	6	-	-

Values are given as mean ± SD or n (%) as appropriate. P values are for a three-way comparison. Bacterial infection refers to any positive blood/sputum/urine culture for pathogenic bacteria within the first month of admission.

*Both were smear positive but culture negative

†Both were culture positive

Seventy five children were investigated for TB and 21/82 (25.6%) were determined to have TB. Eight (10.9%) had bacteriologically confirmed pulmonary TB (one of whom died before the culture flagged positive) while thirteen were clinically diagnosed. Eight were treated empirically after admission, while five were on treatment at enrolment but had no positive TB results on record. All six culture-positive TB isolates were fully sensitive in vitro.

62 patients had sputum samples, while the remaining 13 had gastric aspirates. The mean ages of those with sputum or gastric aspirate samples did not differ significantly (25 versus 21 months, p = 0.7). No gastric aspirate samples tested positive for TB; two empirically-treated patients had had gastric aspirate samples tested. Xpert was employed in 45 patients, but was only positive in two, both of whom were subsequently culture positive. Of the 37 patients enrolled before Xpert was widely available, five had confirmed TB and seven were empirically treated, versus three confirmed and six empirically treated after Xpert was introduced (p=0.199). Thus, the introduction of Xpert did not appear to affect the rates of empiric treatment for TB in smear-negative patients, though it should be underlined that this study was carried out while local clinicians were still familiarising themselves with Xpert.

One empirically-treated child was suspected of having TB meningitis clinically and on cerebrospinal fluid analysis (leukocytosis and elevated protein levels), but was TB culture

negative from all specimens. The remainder of those commenced on empiric treatment at our centre were suspected of having pulmonary TB. Prior to their admission to KEH, two children were on treatment for suspected pulmonary TB, two for suspected disseminated TB, and one for suspected abdominal TB.

5.4.2 Regression Analysis

Fifty-three children had sufficient data for inclusion in the multivariate analysis, including 7 of those with proven TB (the eighth child, who was smear-positive but culture-negative, was excluded for lack of a full blood count result). Results of the regression analysis are presented in Table 18. While leukocytosis was associated with a slight increased risk of TB in the final model (aRR 1.81, 95% CI 1.067—1.308), thrombocytosis was unexpectedly associated with a reduced risk (aRR 0.868, 95% CI 0.774—0.974). The diagnosis of a culture-proven bacterial infection during the admission was associated with a reduced risk of TB (aRR 0.856, 0.748—0.979). Sputum sampling (as opposed to gastric washings) was associated with a 13.4% increased risk of subsequent diagnosis of TB (95% CI 2.1%—26%). While allocation to the “delayed ART” group was associated with a 13.8% increased risk of TB in the full model, this association disappeared when the parsimonious model was created and it was omitted from the final model. There was a small reduction in risk for children with CD4 percentages between 20—25% (aRR 0.864, 95% CI 0.765—0.976 with CD4 percentage >25% used as the reference category), but no other CD4 values were associated with either increased or decreased risk.

Table 18. Results of regression analysis. Data are presented as: (a) n (%); (b) median (IQR); (c) RR (95% CI)

Risk factor	TB	No TB	Unadjusted ^c	p (Wald)	Fully adjusted ^c	p (Wald)	Parsimonious adjusted ^c	p (Wald)
Total (n = 53)	7	46						
Sex ^a : Male	3 (42.9%)	24 (52.2%)	0.963 (0.82—1.131)	0.645	1.041 (0.933—1.162)	0.467	-	-
Female	4 (57.1%)	22 (47.8%)	1	-	1	-	-	-
Delayed ART ^a	5 (71.4%)	22 (47.8%)	1.101 (0.942—1.286)	0.229	1.138 (1.003—1.291)	0.044	-	-
Early ART	2 (28.6%)	24 (52.2%)	1	-	1	-	-	-
Oedema present ^a	3 (42.9%)	11 (23.9%)	1.101 (0.904—1.341)	0.337	1.052 (0.904—1.247)	0.464	-	-
No oedema	4 (57.1%)	35 (76.1%)	1	-	1	-	-	-
Hb <10g/dL ^a	3 (42.9%)	12 (26.1%)	0.921 (0.761—1.114)	0.397	0.870 (0.740—1.023)	0.092	0.875 (0.756—1.014)	0.075
Hb ≥10g/dL	4 (57.1%)	34 (73.9%)	1	-	1	-	1	-
WBC >12 x 10 ⁹ ^a	7 (100%)	29 (63%)	1.194 (1.072—1.331)	0.001	1.199 (1.057—1.359)	0.005	1.181 (1.067—1.308)	0.001
WBC ≤12 x 10 ⁹	0	17 (37%)	1	-	1	-	1	-
Platelets ≥400 x 10 ⁹ ^a	1 (14.3%)	14 (30.4%)	0.921 (0.789—1.076)	0.299	0.825 (0.730—0.932)	0.002	0.868 (0.774—0.974)	0.016
Platelets <400 x 10 ⁹	6 (85.7%)	32 (69.6%)	1	-	1	-	1	-
Albumin <25g/L ^a	5 (71.4%)	26 (56.5%)	1.065 (0.91—1.245)	0.434	0.940 (0.788—1.120)	0.486	-	-
Albumin ≥25g/L	2 (28.6%)	20 (43.5%)	1	-	1	-	-	-
CD4 <15% ^a	4 (57.1%)	18 (39.1%)	1.064 (0.845—1.338)	0.599	1.039 (0.899—1.201)	0.604	1.082 (0.927—1.264)	0.317
CD4 15—20%	2 (28.6%)	12 (26.1%)	1.029 (0.805—1.314)	0.821	0.914 (0.760—1.100)	0.343	0.985 (0.822—1.181)	0.87
CD4 20-25%	0	8 (17.4%)	0.900 (0.748—1.083)	0.264	0.806 (0.690—0.942)	0.007	0.864 (0.765—0.976)	0.019
CD4 >25%	1 (14.3%)	8 (17.4%)	1	-	1	-	1	-
Any positive bacterial culture ^a	2 (28.3%)	24 (52.2%)	0.909 (0.777—1.062)	0.229	0.844 (0.752—0.948)	0.004	0.856 (0.748—0.979)	0.024
No positive bacterial culture	5 (71.7%)	22 (47.8%)	1	-	1	-	1	-
Sampling site: Sputum ^a	7 (100%)	37 (80.4%)	1.159 (1.056—1.272)	0.002	1.115 (0.989—1.256)	0.076	1.134 (1.021—1.260)	0.019
Gastric washings	0	9 (19.6%)	1	-	1	-	1	-
Age (months) (Median, IQR) ^b	15 (8—42)	18 (6.75—27)	1.001 (0.998—1.005)	0.463	1.002 (0.999—1.005)	0.151	-	-

5.5 Discussion

In our prospective cohort of 82 HIV-infected South African children, admitted to a university teaching hospital for the management of complicated SAM, we found a 25.6% incidence of TB, with bacteriological confirmation in 38% of cases ($n = 8/21$).

Children with HIV often present with paucibacillary TB disease with poor sensitivity on culture and Xpert compared to adults, difficult sample acquisition (particularly in the case of extrapulmonary TB and young children) and malnutrition is a component of most clinical diagnostic scoring systems, rendering these less reliable in a population of malnourished children (21). We found few associations between routine laboratory parameters (thrombocytosis, white blood cell monocytosis and high globulin fraction) and culture-proven TB. Ultimately our best regression model was still not a particularly good fit for the data, likely due to unmeasured confounders and a lack of clinical and radiological details, and the associations we identified were not strong enough to be useful in a predictive model. Further studies are required to identify biomarkers of active TB that are reliable in severely-malnourished, HIV-infected children (22).

In the regression model, sputum sampling was associated with a diagnosis of TB when compared with gastric aspirates, which is consistent with previous studies showing superior sensitivity of sputum sampling (23). Some of the other associations that we identified require a nuanced interpretation. The association of delayed ART and TB was seen in the fully adjusted model but not the final parsimonious model, and is not likely to be a true association, as all but one positive sputum sample were acquired during the first week of admission—i.e. early ART would not have prevented these diagnoses of TB. In addition, randomisation was stratified by TB status at enrolment. Possibly this finding indicates sampling bias by clinicians in this open-label study. The strongest conclusion one could draw is that the lack of increased risk of TB following early ART assuages concerns regarding immune reconstitution inflammatory syndrome (IRIS) reactions in these children (although, of note, Bacille Calmette-Guérin IRIS did occur in one child in the early ART group; this child was not classified as having TB in our study).

Culture-proven bacterial infection was associated with a 14.4% reduced risk of culture-proven TB—not only is it exceedingly unlikely that bacterial infections are protective against mycobacterial infections, but multiple simultaneous opportunistic infections are in fact expected in severely immunosuppressed children. It is more likely that treatment for bacterial infections led to false-negative TB samples, or that treatment for TB resulted in false-negative bacterial cultures. Broad-spectrum antibacterials—empiric or otherwise— (including Tazobactam, amikacin, ciprofloxacin and carbapenem) were frequently administered to the children in this study, as SAM is frequently associated with infections at time of presentation,

and hospital-acquired infections are also common in this patient group.(58) While the local formulary dictates that quinolones only be prescribed if indicated by antibiotic susceptibility profiles or on the advice of the infectious diseases service, aminoglycosides are frequently employed, as were carbapenems (24), both of which have activity against TB (25). We were unable to access the original charts for most patients, meaning that we could not correlate actual antibiotic prescriptions with the likelihood of a TB diagnosis. However, it is reasonable to assume that TB diagnoses could be masked by the treatment of other infections (or vice versa) in severely unwell children with HIV and SAM, or not considered when a child with SAM presents to hospital with a sepsis-like syndrome and therefore it would be advisable to include TB investigation as part of the standard admission workup in order to minimize the risk of false negatives in the acute phase of malnutrition management.

This is, to our knowledge, the first study describing TB in children who all have both HIV and SAM. Looking beyond the multivariate model in a subset of patients, there were 13 additional patients in this study who were empirically treated for TB. The true rate of TB in children with SAM and HIV remains unquantifiable as long as so many cases remain unconfirmed. Table 19 summarises a sample of studies of such populations (26,27): while the rate of confirmed TB in children with SAM appears to rise in parallel with increasing rates of HIV (allowing for local variation in baseline TB rates), rates of empiric treatment vary substantially. Thus, it is impossible to assess the separate contributions of HIV and SAM to the pathogenesis of TB in children, though Table 19 does suggest that HIV is a key risk factor for TB in SAM—with increasing numbers of malnourished children now having HIV as an underlying cause, it is important for clinicians to particularly consider TB in such cases. Our own study shows that, despite the inherent challenges, microbiological confirmation of suspected pulmonary TB is possible in severely malnourished children with HIV.

Table 19. Examples of other studies of children admitted for the management of SAM in different sub-Saharan African settings

These data suggest that, as rates of HIV increase, so too do rates of bacteriologically confirmed TB in children with SAM—however, rates of empiric treatment differ markedly between studies and confound accurate assessment

Study	Year	Setting	Number with SAM	Prevalence of HIV ^a	Prevalence of confirmed TB ^a	Prevalence of empiric TB treatment ^a	Overall rate of TB treatment ^a
Cartmell (Part 1) ²⁶	1983	Maputo, Mozambique	833	0	53 (6.4%)	29 (3.5%)	81 (9.7%)
Cartmell (Part 2) ²⁶	2001	Maputo, Mozambique	558	65 (29.3%)*	78 (14%)	14 (2.5%)	92 (16.5%)
De Maayer ²⁷	2011	Johannesburg, South Africa	113	58 (51%)	3 (2.6%)	29 (25.7%)	32 (28.3%)
This study	2016	Durban, South Africa	82	82 (100%)	8 (9.8%)	13 (15.9%)	21 (25.6%)

(a) n (%)

*51 with an AIDS-defining-illness, 14 with confirmed HIV. An additional 30 had suspected HIV but this was not confirmed (not included in this table)

Other studies in Africa have started with a diagnosis of TB and looked backwards for associations with malnutrition and/or HIV, usually finding strong and independent associations with both of these risk factors and TB mortality (12,13,28). The only other studies that we can find that began with malnourished children and looked for associations with TB were carried out in South Asia, where paediatric HIV prevalence is far lower, and differ in many important methodological factors from our study (29,30). One such study examined 405 severely malnourished Bangladeshi children with respiratory symptoms and radiographic pulmonary infiltrates: 7% had confirmed TB and a further 16% were treated based on clinical suspicion (30). HIV prevalence was not determined, but was known to be rare in that locale. While this study is important in raising awareness of TB mimicking acute pneumonia in children with SAM, the TB prevalence in a select population with radiologic changes will obviously be higher than the TB prevalence in malnourished children in general.

Strengths of our study include prospective recruitment of patients and access to tertiary-level diagnostic facilities. Limitations include the relatively small number of patients, missing data, practice changes during the course of the trial (e.g. the introduction of Xpert) and the single-centre setting, limiting the generalisability to other settings in sub-Saharan Africa and beyond. Due to the retrospective nature of the study, poor availability of patients' original hospital files meant that we lacked data regarding clinical findings, radiographs and tuberculin skin tests, which would have been a useful addition to our analysis. We considered our two patients who were smear-positive but culture negative to have confirmed TB, in keeping with the WHO classification (20), but these may have been false positives due to non-tuberculous mycobacteria—equally, we considered patients who started treatment in other centres to be empirically-treated, when they may in fact have had a confirmed diagnosis which was not identifiable on the national laboratory online system.

5.7 Conclusions

TB is common in HIV-infected children with severe acute malnutrition. While microbiological confirmation of the diagnosis is feasible, empiric treatment remains common in this patient group, possibly influenced by suboptimal testing (gastric aspirates as opposed to induced sputum) and false negative TB diagnostics secondary to antibacterial therapy. Future studies should focus on diagnostic strategies that are sufficiently robust for this important, vulnerable population of children in resource-poor settings. With SAM being increasingly seen in conjunction with HIV in sub-Saharan Africa, it is important that TB diagnosis and treatment be integrated into the programmatic management of these conditions.

5.8 Acknowledgements

We thank all of the participants and their carers. In addition, we are grateful to the members of the LivTB research group in Liverpool for helpful discussions and to Ms Thobekile Sibaya for administrative support.

5.9 Further Reading

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BRIDGING TEXT

Delaying ART initiation until after nutritional recovery resulted in improved rates of WAZ and HAZ change in severely malnourished HIV-infected children (chapter 2). However, the proposed mechanism for this difference (altered lopinavir pharmacokinetics) was found not to be influenced by the delay in ART initiation (chapter 3). In the literature review we described the influence of intestinal microbiota on nutrition and propose an alternative mechanism for this difference. In the following chapter the influence of malnutrition and HIV infection on bacterial translocation, immune activation and immune exhaustion is explored. The effect of malnutrition on these factors, following ART initiation in severely malnourished and non-malnourished HIV-infected children is further explored.

CHAPTER 6

MALNUTRITION INCREASES MICROBIAL TRANSLOCATION, SYSTEMIC IMMUNE ACTIVATION AND IMMUNE EXHAUSTION AND IMPAIRS IMMUNE RECOVERY IN HIV-INFECTED CHILDREN

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6.1 Abstract

Objectives

This study aimed to assess the impact of malnutrition on immune pathogenesis and treatment outcomes in HIV-infected children.

Design

We studied markers of microbial translocation (16sDNA), intestinal damage (iFABP), monocyte activation (sCD14), T-cell activation (CD38, HLA-DR) and immune exhaustion (PD1) cross-sectionally in a cohort of 32 HIV-infected and 15 HIV-uninfected malnourished children compared to 41 HIV-infected and 19 HIV-uninfected children with normal nutritional status. In the longitudinal part of this study we assess the effects of 48 weeks of antiretroviral therapy on these markers and investigated their association with different treatment outcomes in HIV-infected children.

Methods

Plasma levels of 16sDNA, iFABP and sCD14 were measured by quantitative real time PCR, ELISA and Luminex, respectively. T-cell phenotype markers were measured by flowcytometry. Multiple regression analysis was performed using Generalised Linear Models (GLMs) and the Least Absolute Shrinkage and Selection Operator (LASSO) approach for variable selection.

Results

Microbial translocation, T-cell activation and exhaustion were increased in HIV-infected and HIV-uninfected malnourished children compared to HIV-uninfected children with normal nutritional status. Malnutrition, age, microbial translocation, monocyte and CD8 T-cell activation were independently associated with decreased rates of CD4% immune recovery after 48 weeks of ART. Microbial translocation, immune activation and exhaustion remained elevated despite viremic suppression after 48 weeks of antiretroviral therapy.

Conclusions

Malnutrition is associated with microbial translocation, immune activation, immune exhaustion and impaired immune recovery in HIV-infected children on ART.

6.2 Introduction

Malnutrition was estimated to be the underlying cause for 45% of deaths in children less than five years of age in 2011 globally (1). The prevalence of malnutrition in HIV-infected children is as high as 40% making clinical management of this vulnerable population even more challenging (2,3). Co-occurrence of severe acute malnutrition (SAM) and HIV results in increased morbidity and mortality largely due to increased incidence and severity of concurrent infections (4–6). Immunological alterations such as systemic inflammation and impaired cellular immune responses have been attributed to malnutrition (7) that could explain this increased susceptibility to infection especially when exacerbated by HIV-infection, but the underlying mechanisms remain largely unresolved.

Malnutrition is characterized by functional and structural alterations in the intestinal mucosa associated with chronic intestinal inflammation (8). Direct effects of protein malnutrition (9) and perturbations in the gut microbiome (10) that are only partially restored after nutritional interventions (11,12) have been proposed as a primary cause for persistent intestinal inflammation and epithelial damage. In HIV-infection breakdown of the intestinal barrier, depletion of gut-resident CD4+ T-cell populations and mucosal immune dysregulation result in microbial translocation that drives systemic immune activation (13). Chronic immune activation is a hallmark of disease progression in HIV-infected children and results in accelerated loss of CD4 T-cells, immune-dysregulation and immune exhaustion (14).

It is not known whether SAM exacerbates the degree of microbial translocation, leading to increased immune activation and immune exhaustion in HIV-infected children and whether this is modulated by ART. We thus sought to describe and compare levels of microbial translocation, immune activation and exhaustion in a cohort of HIV-uninfected and HIV-infected ART-naïve children presenting with SAM and after one year of ART. We hypothesize that (i) malnutrition increases microbial translocation, immune activation and immune exhaustion in HIV-infected and –uninfected children; (ii) malnutrition, microbial translocation and immune activation are associated with detrimental treatment outcome in HIV-infected children on ART. Data from this study may add to the currently limited knowledge guiding management of HIV and SAM and shed light on the extent of microbial translocation in this population and potential ways of curbing this process.

6.2.1 Materials and methods

6.2.1.1 Study subjects and procedures

Study participants were recruited from pediatric clinics and wards at the Ithembalabantu Clinic, Umlazi, and the King Edward VIII Hospital, Durban, South Africa. We studied four different groups of children: (i) 32 ART-naïve HIV-infected children with severe acute malnourishment (HIV+SAM+); (ii) 41 ART-naïve HIV-infected children without SAM (HIV+SAM-); (iii) 15 HIV-uninfected children with SAM (HIV-SAM+); (iv) 19 HIV-

uninfected children without SAM (HIV-SAM-). HIV+SAM+ children were recruited as part of the previously described MATCH cohort¹⁵. HIV-uninfected siblings from HIV+SAM- children were recruited for the HIV-SAM- control group.

SAM was defined based on 2009 WHO criteria as presence of: (i) weight-for-height more than three SD below the **median**, (ii) mid-upper arm circumference < 115mm or (iii) the presence of bilateral lower extremity pitting edema of nutritional origin. The WHO anthro software package 3.2.2 for SPSS (<http://www.who.int/childgrowth/software>) was used to calculate anthropometric z-scores.

Management of SAM was initiated according to WHO recommendations. All children with SAM received antibiotics (Penicillin and Gentamicin) and antihelminthics (albendazole). HIV+SAM+ children additionally received **Cotrimoxazole** prophylaxis. All HIV-infected children were initiated on ART according to the current South African guidelines using a combination of abacavir and lamivudine plus either efavirenz or ritonavir boosted lopinavir. HIV+SAM+ children were initiated on ART after a median time of 11 days following hospitalization. Baseline blood samples were taken on the same day before the first dose of ART was given. HIV-positive children were followed up at week 12, 24 and 48. All children were screened for active Tuberculosis disease and if indicated treated according to national guidelines.

Written informed consent was obtained from caregivers for all study participants and, additionally, assent to participate in the study was given directly by children in the appropriate age groups. The study was reviewed and approved by the University of KwaZulu-Natal Ethics Review Board, Columbia University Institutional Review Board and the Oxford Research Ethics Committee.

6.2.1.2 CD4 count and viral load measurements

Viral load levels were determined using the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2.0 by Roche. CD4+ T cell counts and percentage were measured by flow cytometry.

6.2.1.3 Quantification of plasma biomarkers

Plasma levels of sCD14 were quantified using a luminex kit (R&D systems, Minneapolis, MN) at a 100-fold dilution in duplicate as per manufacturer's recommendations. Plasma concentrations of intestinal fatty acid-binding protein (iFABP) were quantified using an ELISA kit (R&D systems, Minneapolis, MN) in duplicate at a 1:10 dilution according to the manufacturer's instructions. Results were expressed in pg/ml.

6.2.1.4 Real-time PCR quantitation of 16S rDNA in plasma

To minimize the risk of contamination blood samples were obtained using standard aseptic techniques following cleansing of the skin with 2% chlorhexidine/alcohol solution. Subsequently, all samples were handled in laminar flow cabinets under sterile conditions. Total DNA was extracted from 200µl plasma using the column-based QIAmp DNA mini kit

(Qiagen). 16S rDNA was amplified using the following published primers¹⁶: 5'-AGAGTTTGATCCTGGCTCAG -3' (forward) and 5'-CTGCTGCCTCCCGTAGGAGT -3' (reverse). Each PCR comprised 3.5 mmol/μL MgCl₂, 0.125 pmol/μL of each primer, 5 μL of SYBR Green I Master Mix (2X) (Roche), 2 μL of DNA, and water to 10 μL. Reactions were run in triplicate on a Roche LightCycler 480 version 1.5 as follows: 1 cycle at 95°C for 10min, followed by 45 cycles at 95°C for 30s, at 60°C for 30s, and at 72°C for 30s. A standard curve was created from serial dilutions of DH5α bacterial DNA containing known copy numbers of the template calculated by molecular weight as described previously¹⁷. Individual samples were then analyzed against the standard curve and 16s rDNA copy numbers per microliter of plasma were calculated.

6.2.1.5 Surface phenotypic staining and flowcytometry

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation and used for staining with fluorochrome-conjugated monoclonal antibodies against cell surface markers for T-cell activation, exhaustion and memory differentiation. Briefly, isolated PBMCs were washed in PBS and incubated with titrated concentrations of the following antibodies in a staining volume of 50ul: CD3-BV605 (BD), CD4-V450 (BD), CD8-HV500 (BD), CD38-PECy7 (BD), CCR7-PE (R&D), HLA-DR-FITC (BD), CD45RA-AlexaFluor700 (Biolegend) and PD1-APC (eBioscience). Near-infra red Live/dead marker (Invitrogen) was used to exclude non-viable cells. Fluorescence minus one (FMO) staining was used to determine the cut-off for PD1-high cells. After 20 minutes incubation at room temperature cells were washed twice in PBS and resuspended in PBS with 2% paraformaldehyde (PFA). Data was acquired on a LSR-II driven by FACS DiVa software (BD) and analyzed using FlowJo software v8.8.6 (Treestar, Ashland, OR).

6.2.1.6 Statistical Analysis

Baseline characteristics were compared between study groups using Wilcoxon rank sum tests. Spearman correlations were used to explore bivariate associations. Differences in mortality rates were calculated using Chi-Square tests. These statistical analyses were performed using GraphPad Prism version 6.0c (GraphPad Software Inc., La Jolla, CA, USA).

Multiple regression analysis was used to assess associations between clinical and immunological covariates with CD4 T-cell immune activation in a Gaussian generalized linear model (GLM) with log link function.

For the analysis of associations between baseline predictors and treatment outcomes a model with favorable properties in regards of model complexity and statistical inference is needed due to the relatively small sample size in relation to the number of regarded covariates. Moreover, because of a high degree of correlation between some of the co-variates the model should be able to handle a certain level of multicollinearity. We therefore used an approach for variable selection applying the Least Absolute Shrinkage and Selection Operator (LASSO)

principle on scaled covariates (18). Subsequently, on the set of covariates selected by LASSO an un-regularized conventional GLM was fitted in order to obtain un-shrunked coefficient estimates together with standard errors. For better comparison of coefficient estimates between the LASSO and GLM all covariates have been scaled. For the regression analysis of the binary outcome ‘W48 virologic suppression’ a binomial GLM with logit link function was used. For the continuous outcome ‘CD4% immune recovery’ a Gaussian GLM with log link function was chosen. ‘Virologic suppression’ was defined as viral load levels below limits of detection at the 48week study visit. ‘Immune recovery of CD4%’ was defined as the difference of CD4% at the 48week study visit and baseline. The statistical software R (R Development Core Team, 2014) was used for all computations applying the R-packages *glm* and *glmnet* (19).

6.3 Results

6.3.1 Baseline characteristics

Clinical characteristics of each study groups are summarized in Supplementary Table 20. Among HIV-infected children, those with SAM were younger than their counterparts without SAM reflecting accelerated disease progression in this population that results in earlier clinical presentation. Children with SAM, regardless of HIV status, were generally stunted with low height for age z-scores (haz) and showed signs of mixed, i.e. chronic and acute, malnutrition as expressed in low weight for age (waz) combined with low weight for height z-scores (whz). HIV+SAM- children were mildly stunted with low haz scores, but normal waz and whz scores. Prevalence of active tuberculosis (TB) disease was higher in malnourished children with 15 cases out of 32 HIV+SAM+ children and only 5 out of 41 HIV+SAM- children at baseline (p=0.0003). TB therapy was initiated as per current South African guidelines.

Table 20. Clinical characteristics of study groups defined by HIV-status and severe acute malnutrition (SAM) at baseline.

Baseline characteristics				
Characteristics	HIV+ SAM+ (n=32)	HIV+ SAM- (n=41)	HIV- SAM+ (n=15)	HIV- SAM- (n=19)
Age (years)	1.6 (0.7 - 3.4)	8.2 (4.5 - 10.7)	0.9 (0.5 - 1.5)	9.1 (6.8 - 12.8)
Male sex	18 (56%)	26 (63%)	7 (47%)	9 (47%)
CD4+ T-cell count, cells/mm ³	541 (149 - 1167)	412 (212 - 875)	N/A	N/A
CD4%	14.9 (8.5 - 20)	18.0 (9 - 24.5)	N/A	N/A
HIV RNA level, copies/mL	1.2x10 ⁶ (5.9x10 ⁵ - 7.3x10 ⁶)	1.4x10 ⁵ (3.3x10 ⁴ - 5.9x10 ⁵)	N/A	N/A
Active Tuberculosis-disease	15 (47%)	5 (12%)	0	0
Height for age z score	-3.3 (-4.7 - -2.4)	-2.0 (-2.8 - -1)	-2.1 (-3.6 - -1.2)	-0.2 (-1.6 - 1)
Weight for age z score	-3.4 (-4.5 - -3)	-0.9 (-1.9 - -0.3)	-1.8 (-4 - -1.4)	0.0 (-0.5 - 0.9)
Weight for height z score	-1.9 (-5.3 - -0.6)	0.5 (-0.5 - 1.4)	-1.2 (-3.3 - 0)	0.9 (0.2 - 2)

Median values with interquartile range or number of study subjects with percentage are shown for each clinical parameter by study group.

6.3.2 Malnourishment and HIV-infection have differential effects on microbial translocation, intestinal damage and monocyte activation

Microbial translocation, as measured by levels of bacterial 16sDNA in plasma, was significantly increased in all groups of HIV-infected and malnourished children compared to healthy controls (Fig 12a). Children with SAM had higher plasma levels of iFABP, a marker of intestinal damage, compared to children without SAM, regardless of HIV status. The marker of monocyte activation and inflammation differed by HIV but not by SAM status: sCD14 levels were higher in the HIV-infected compared to HIV-uninfected group but SAM did not result in higher sCD14 in the HIV-infected group (Fig 12b,c). In HIV-infected children, high iFABP levels were associated with stunting (low height for age) and underweight (low weight for age) (Fig 12d). In the HIV+SAM- group, iFABP correlated with sCD14 and sCD14 inversely correlated with CD4% (Fig 12e).

Fig 12

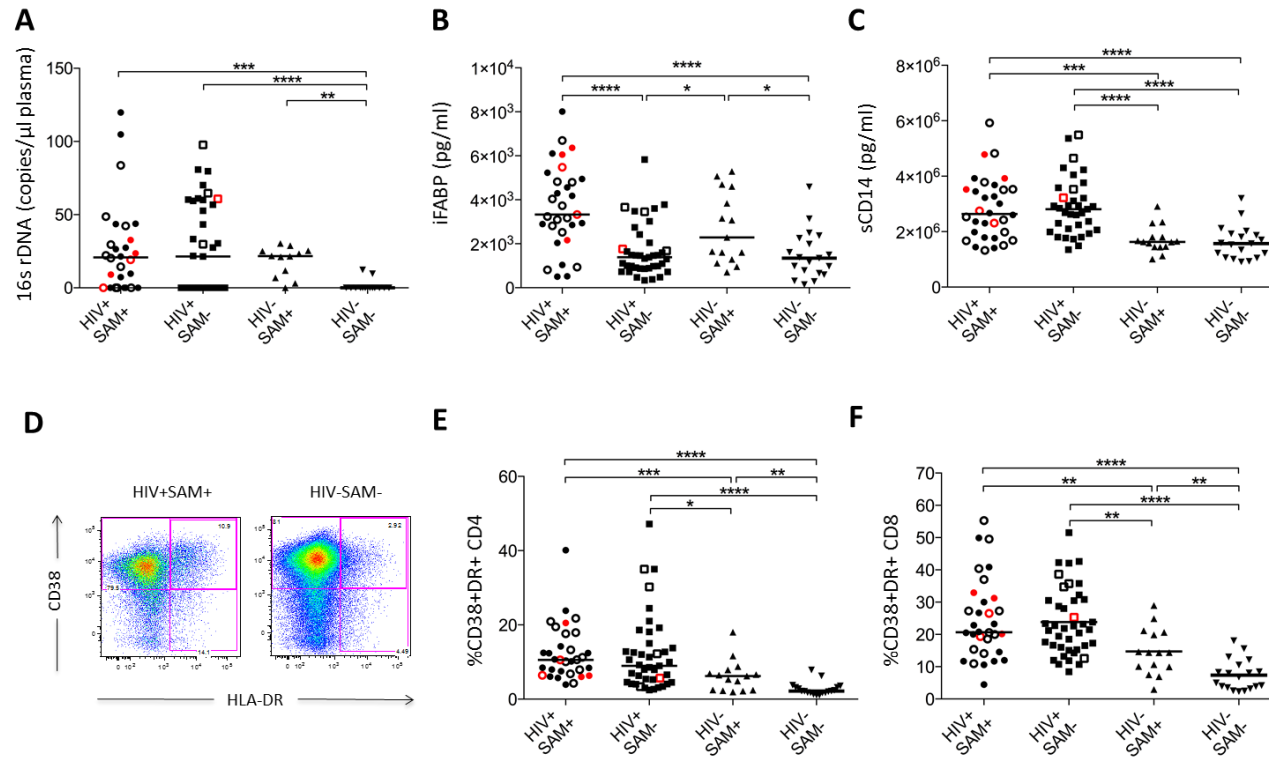


Figure 12. Plasma biomarkers by study group.

A-C: Plasma levels of 16sDNA (A), iFABP (B) and sCD14 (C) in children stratified by HIV-status and presence of severe acute malnutrition (SAM) at baseline. Open symbols indicate active tuberculosis disease; red symbols indicate children that passed away during the study period. Medians are shown as horizontal bars. Mann-Whitney test was used to determine statistical significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). D+E: Correlations between plasma markers and clinical parameters in all HIV-positive children (D) and HIV-positive children without SAM (E). Statistical significance was calculated using Spearman tests.

6.3.3 Immune activation is increased by malnutrition and HIV-infection

We next investigated T-cell activation by measuring CD38 and HLA-DR expression in CD4⁺ and CD8⁺ T-cells. Levels of immune activation in the CD4⁺ and CD8⁺ T-cell compartment were highest in the HIV-infected study groups, but also significantly elevated in HIV-uninfected children with SAM compared to healthy controls (Fig 13a,b,c). In regression analysis SAM (β -coefficient=0.48, p-value=0.0083) and HIV-infection (β -coefficient=1.04, p-value<0.0001) were independently associated with increased CD4⁺ T-cell activation controlling for age, sex and TB-status (Supplementary Table 21).

Fig 13

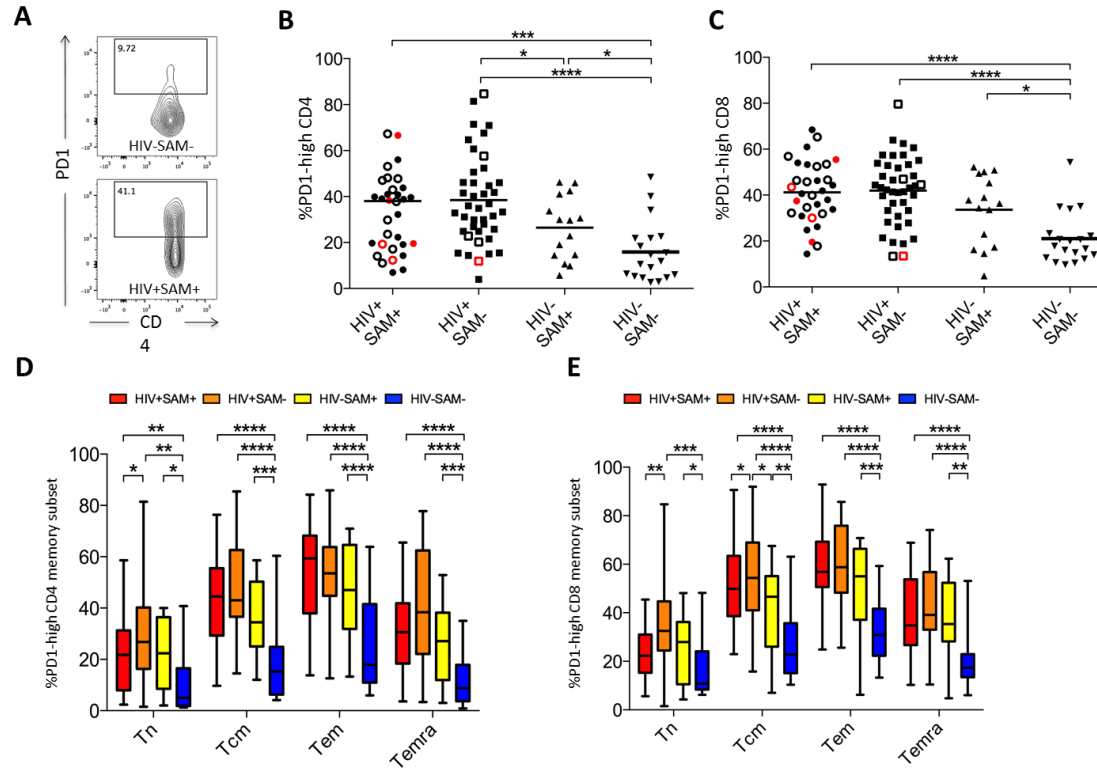


Figure 13. T-cell activation by study group.

A: Representative CD4+ T-cell immune activation FACS data from an HIV-positive child with severe acute malnutrition (HIV+SAM+, top) and a healthy control (HIV-SAM-, bottom). B+C: Immune activation of CD4+ and CD8+ T-cells in children stratified by HIV-status and nutritional state. Open symbols represent children with active tuberculosis disease; red symbols represent children that passed away during the study period. Mann-Whitney test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). D: Correlation between markers of microbial translocation and CD4 T-cell activation for all groups. E-F: Correlation between CD4+ T-cell activation and clinical parameters for all HIV-positive children. Spearman r .

Table 21: Effect of clinical and immunological covariates on CD4+ T cell activation

Results are shown for multiple regression analysis with ‘CD4+ T cell activation’ as dependent variable and a set of clinical covariates. ‘HIV-infection’ and ‘SAM’ (severe acute malnutrition) are independently associated with higher levels of ‘CD4+ T cell activation’.

Multivariate analysis of clinical and immunological factors associated with CD4+ T-cell activation		
Co-Variable	GLM	
	β -coefficient	p-value
HIV-infection	1.04	<0.0001
SAM	0.48	0.008
Age	0.022	0.21
Sex (male)	-0.036	0.79
Active TB	0.115	0.54

CD4+ T-cell activation correlated with higher levels of 16sDNA and sCD14 (Fig 13d). In HIV-infected children, high levels of CD4+ T-cell activation were associated with stunting (low height for age) and underweight (low weight for age) (Fig 13e). T-cell activation, especially of CD4+ T-cells, showed strong correlations with clinical markers of disease progression (Fig 13f), highlighting the central role of systemic immune activation in the pathogenesis of HIV-infection.

6.3.4 PD1 expression is increased in malnourished and HIV-infected children and associated with immune activation

We next hypothesized that higher levels of immune activation in malnourished and HIV-infected children would be associated with increased immune exhaustion and studied PD1 expression, a marker of immune exhaustion (20). PD1 surface expression was increased in CD4+ and CD8+ T-cells in all malnourished and HIV-infected study groups (Fig 14a, b, c).

Because PD1 expression is commonly higher in T-cells with a more terminally differentiated memory phenotype (21), we next assessed PD1 expression on different memory subsets. PD1 expression was highest in the CD4+ and CD8+ T effector memory populations (Tem) and similarly increased in malnourished and HIV-infected children compared to healthy controls (Fig. 14d, e). We observed strong correlations between T-cell activation and PD1 expression (Fig 14f) reflecting the overlap in the regulation of cellular activation and expression of co-inhibitory molecules such as PD1.

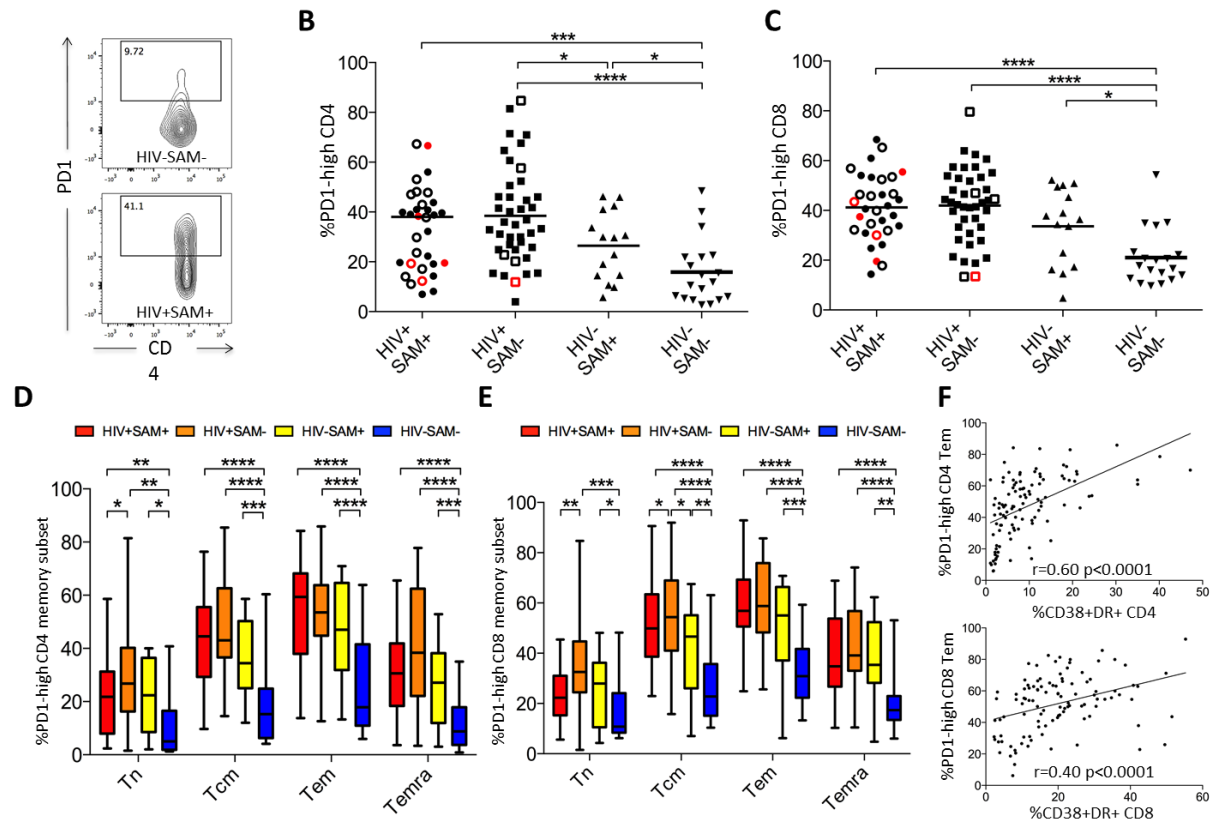


Figure 14. PD1 expression by study group.

A: CD8+ T-cell PD1 FACS data showing an FMO control (top) and a representative HIV+SAM+ sample (bottom). B+C: PD1 expression in CD4+ and CD8+ T-cells by study group. Open symbols indicate active TB disease; red symbols indicate children that passed away during the study period. Mann-Whitney test (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001). D+E: PD1 expression on CD4 and CD8 T-cell memory subsets defined by CD45RA and CCR7 expression (T naive: CD45RA+CCR7+, T central memory: CD45RA-CCR7+, T effector memory: CD45RA-CCR7-, T effector memory RA: CD45RA+CCR7-). Mann-Whitney test (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001). F: Correlation between T-cell activation and PD1 expression. Spearman test

6.3.5 Severe acute malnutrition is associated with higher mortality in HIV-infected children starting ART

All HIV-infected children were initiated on ART according to the current South African guidelines. During the 48week follow up period of this study, 5 out of 32 children in the HIV+SAM+ group and 1 out of 41 children in the HIV+SAM- group deceased despite optimal clinical care. The higher mortality rate of children with SAM was statistically significant ($p=0.021$, Chi-square test, data not shown) and independent of active TB disease. Excluding all TB cases from the analysis, 3 out of 17 children in the HIV+SAM+ group died compared to 0 out of 36 children in the HIV+SAM- group ($p=0.0047$, Chi-square test).

6.3.6 Malnutrition, microbial translocation and immune activation are associated with detrimental treatment outcome in children initiating ART

We next evaluated if any clinical and immunological parameters at baseline were predictive of treatment outcomes in HIV-infected children after 48weeks of ART.

For the 48 HIV-infected children who completed the follow up period with available data for all parameters at baseline we first assessed which variables were associated with virologic suppression at week 48. Because of the fairly large number of covariates compared to the relatively low number of cases studied here, we used the LASSO approach for variable selection with subsequent confirmation of the selected co-variables in a generalized linear model (GLM). Active TB disease and high levels of sCD14 and 16sDNA at baseline were independently associated with virologic failure at W48 (Table 22).

Table 22: Effect of clinical and immunological predictors on treatment outcomes.

In the left panel, results are shown for analysis of the clinical outcome ‘W48 virologic suppression’ (i.e. viral load below detectable limits at the 48week study visit). LASSO selected three predictor variables, ‘active TB (tuberculosis) disease’, ‘sCD14’ and ‘16sDNA at baseline’, to be negatively associated with successful virologic suppression out of a set of 14 predictor variables. These results of the LASSO were confirmed using ‘active TB disease’, ‘sCD14’ and ‘16sDNA’ in a generalised linear model. In the right panel, results are shown for analysis of recovery of CD4% from baseline at week 48, i.e. the difference between CD4% at the week48 study visit and baseline. LASSO selected 8 predictors of which ‘sCD14’, ‘16sDNA’, ‘SAM’ (severe acute malnutrition), ‘Age’, ‘CD8 T cell activation’ and ‘CD4% at baseline’ could be confirmed in a generalised linear model to be negatively associated with immune recovery at a statistically significant level. For better comparison of beta-coefficients, scaled data was used for all models.

Predictors associated with HIV treatment outcomes							
Co-Variable	W48 virologic suppression			Co-Variable	CD4% immune recovery		
	LASSO β-coefficient	GLM β-coefficient	p-value		LASSO β-coefficient	GLM β-coefficient	p-value
Active TB	-0.67	-1.30	0.005	sCD14	-1.38	-3.10	0.026
sCD14	-0.74	-1.47	0.006	16sDNA	-3.26	-4.32	0.002
16sDNA	-0.50	-1.37	0.025	SAM	-0.99	-4.07	0.019
SAM	0	-	-	Age	-1.84	-3.43	0.037
Age	0	-	-	CD8 activation	-2.74	-4.16	0.012
CD8 activation	0	-	-	CD4%	-5.48	-7.17	<0.0001
CD4%	0	-	-	iFABP	0.49	0.7435	0.61
iFABP	0	-	-	CD4 activation	0.38	1.63	0.30
CD4 activation	0	-	-	Active TB	0	-	-
Viral load	0	-	-	Viral load	0	-	-
Sex (male)	0	-	-	Sex (male)	0	-	-
CD4 count	0	-	-	CD4 count	0	-	-
CD4 PD1	0	-	-	CD4 PD1	0	-	-
CD8 PD1	0	-	-	CD8 PD1	0	-	-
CD4 Tnaïve	0	-	-	CD4 Tnaïve	0	-	-

Subsequently we investigated the relationship of the clinical and immunological parameters at baseline with the rate of immune reconstitution as measured by recovery of CD4%. We here defined the rate of CD4% recovery, i.e. the difference of CD4% between the week 48 study visit and baseline, as the dependent variable. Out of the set of 14 predictor variables, 8 variables were selected by the LASSO approach. SAM, sCD14, 16sDNA, CD4% and CD8+ T-cell activation at baseline were associated with lower rates of immune recovery at a statistically significant level in the confirmatory GLM (Table 22). These results demonstrate a negative impact of SAM, microbial translocation and systemic immune activation on CD4% recovery in children initiated on ART.

6.3.7 Microbial translocation persists at high levels, but immune activation and immune exhaustion are partially reduced after 48 weeks of ART

We next investigated the effects of ART on microbial translocation, immune activation and immune exhaustion in children with viremic suppression at week 48. Levels of microbial translocation and iFABP remained elevated in HIV-infected children despite 48 weeks of ART (Fig 15a). Monocyte activation, however, as measured by plasma levels of sCD14, decreased significantly to levels similar to healthy controls.

After 48 weeks of ART CD4+ and CD8+ T-cell activation in the central memory compartment (Fig 15b) and to a lesser degree in the effector memory compartment (data not shown) was decreased, but persisted at higher levels than in healthy controls. PD1 expression was significantly reduced (Fig 15c) and the decrease in PD1 expression was strongly correlated to the change of CD4+ T-cell activation (Fig 15d). The decrease in CD4+ T-cell activation was positively correlated with weight for age recovery in HIV-infected children after 48 weeks of ART (Fig 15e), highlighting the link between malnutrition and systemic immune activation.

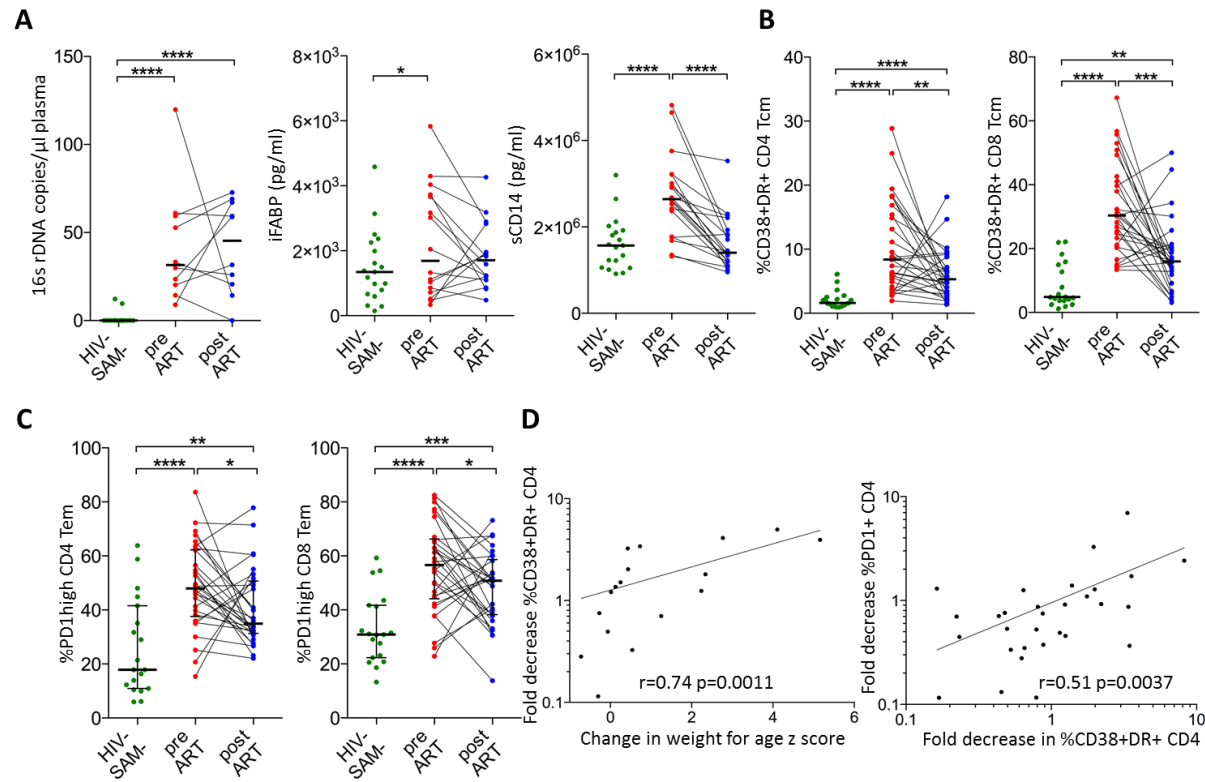


Figure 15. Immune reconstitution in children with virologic suppression after 48 weeks of antiretroviral therapy (ART).

A: Reduction of sCD14 plasma levels in HIV-positive children after 48 weeks of ART (blue) compared to baseline (red) and healthy controls (green). No change in levels of iFABP and 16sDNA. Mann-Whitney test for comparison with HIV-SAM- children and Wilcoxon test for paired data. B: Decrease of CD4 and CD8 T cell activation in the central memory (Tcm) compartment, but persistently elevated levels compared to healthy controls after 48 weeks on ART. C: Reduced PD1 expression of CD4 and CD8 T effector memory (Tem) cells after 48 weeks of ART, but persistently elevated levels compared to healthy controls. D: Correlation between decrease in CD4 T cell activation and weight for age recovery. Correlation between decrease in CD4 T cell activation and CD4 PD1 expression. Spearman r

6.4 Discussion

While microbial translocation, immune activation and immune exhaustion have been identified as key drivers in the pathogenesis of HIV disease, their role in malnutrition remains unclear. We here show that childhood malnutrition is associated with increased microbial translocation, immune activation and immune exhaustion and has a negative impact on treatment outcome in children on ART.

In our study, microbial translocation was not only increased in HIV-infected children as previously reported (22,23), but also in HIV-uninfected malnourished children. Childhood malnutrition leads to alterations of the intestinal microbiome, chronic gut inflammation and structural changes in the epithelium resulting in enteropathy with loss of intestinal barrier functions (7). We here show that plasma levels of iFABP, which is released by dying epithelial cells and used as a plasma marker of intestinal damage (24), were elevated in malnourished children independent of HIV-status indicating structural intestinal damage that predisposes to microbial translocation.

Translocation of luminal bacterial products into the systemic circulation triggers immune activation (13). Bacterial lipopolysaccharide (LPS) activates monocytes by binding to CD14, a co-receptor of the TLR4 complex (25). Upon monocyte activation CD14 is shed from the cell and can be measured as a soluble marker of monocyte activation in the peripheral blood compartment. However, interpretations of sCD14 as an indirect marker of microbial translocation should be taken carefully as monocytes are also activated independent of LPS, e.g. directly by HIV-replication (26), and sCD14 is also secreted as an acute phase protein by hepatocytes (27). In this study, plasma levels of sCD14 were significantly elevated in HIV-positive children consistent with previous reports (22), but not in HIV-negative malnourished children despite increased levels of microbial translocation in this group. Possibly, the degree of microbial translocation in this study group was not sufficient to trigger monocyte activation to levels significantly different to healthy controls. Furthermore, levels of sCD14 were markedly reduced in HIV-infected children after 48 weeks of ART despite ongoing microbial translocation consistent with a previous study of HIV-infected children (28). Taken together the discrepancies between markers of microbial translocation and sCD14 indicate that monocyte activation as measured by sCD14 is not solely dependent on microbial translocation, but is rather part of a complex cascade of generalized hyper-activation of the innate immune system in chronic HIV-infection.

In this study T-cell activation was increased independently by HIV-infection and malnutrition. In pediatric HIV-infection chronic T-cell activation is a key component of systemic immune activation and plays a central role in HIV-pathogenesis (14). Consistent with previous reports (22) we observed increased levels of T-cell activation in HIV-infected children that correlated with markers of disease progression, microbial translocation and monocyte

activation. Earlier studies of malnourished children without apparent infections reported activation of the innate immune system with elevated leukocyte counts and increased acute phase responses⁷. We here show that T-cell immune activation is also increased in HIV-uninfected malnourished children and confirmed an independent effect of SAM on CD4+ T-cell activation controlling for HIV-infection, age, sex and TB-status in a multivariate analysis. Furthermore, the decrease in immune activation was strongly correlated with weight gains in children on ART highlighting the link between malnutrition and systemic immune activation. Taken together, these data show that increased T-cell immune activation forms part of the complex immunological alterations in malnutrition.

Studies of lymphocyte function in malnourished children consistently showed deficits in cellular immunity such as reduced proliferative responses to PHA stimulation (29) and impaired cytokine production upon in vitro stimulation (30–33). These functional deficits resemble the effects of immune exhaustion on T-cell function as previously described in studies of chronic viral infections (LCMV, HIV) and cancer (34). We therefore hypothesized that the alterations in T-cell function observed in malnourished children are associated with immune exhaustion and measured expression levels of PD1, a marker of immune exhaustion (20,35). PD1 expression on T-cells was increased in malnourished HIV-uninfected children to levels similar to HIV-infected children. PD1 levels strongly correlated with T-cell activation at baseline and decreased in parallel with immune activation in children on ART suggesting that immune exhaustion is driven by immune activation as previously hypothesized (36,37). T-cell activation and PD1 expression was significantly reduced in children on ART, but persisted at higher levels than in HIV-uninfected subjects as reported in adults (38–40).

In the prospective part of this study we evaluated the impact of malnutrition, microbial translocation and immune activation on treatment outcomes in HIV-positive children initiated on ART. Consistent with previous reports (4,5), SAM at baseline was highly predictive of all-cause mortality in children on ART. Monocyte activation, microbial translocation and active TB disease were independently correlated with viremic failure at 48 weeks of ART. Monocyte activation, microbial translocation, malnutrition, age and CD8+ T-cell activation at baseline independently had negative effects on immune recovery. These findings are consistent with previous studies in HIV-infected adults that found high levels of T-cell activation (41) and microbial translocation (42,43) to be associated with poor immune recovery.

Our study has a number of important limitations: First, there are considerable age differences between the study groups. This might affect the levels of biomarkers such as iFABP that have been reported to be elevated in early life (28,44). These results should therefore be interpreted carefully and be confirmed in further studies of malnourished infants and children. Second, there is a high degree of comorbidities such as active TB disease in the group of malnourished HIV-infected children with potential impact on the assessed biomarkers. Third,

in the prospective part of the study the number of participants with available data was relatively low and hence we only followed an exploratory, hypothesis building approach in our analysis. These results should be validated in larger cohorts of malnourished HIV-infected children. Finally, due to the observational character of this study we can only describe associations. Causalities between malnutrition, microbial translocation and immune activation could be studied further in animal models.

In summary, we demonstrate that childhood malnutrition is associated with increased microbial translocation, immune activation and immune exhaustion with a negative impact on immune recovery in HIV-infected children on ART. Taken together these findings imply that microbial translocation, immune activation and immune exhaustion are viable targets for additional interventions in malnourished and HIV-infected children.

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CHAPTER 7

SYNTHESIS

7.1 Introduction

Improving child health is a global priority and is encompassed in the Millennium Development Goals. One of these goals was a 2/3 reduction in childhood mortality by 2015 (59). Despite gains in achieving these goals, low and middle income countries (LAMIC) have lagged behind due to the impact of poverty, food insecurity and infectious diseases particularly Human Immunodeficiency Virus (HIV) and Mycobacterium Tuberculosis (TB) on the health of children (60–64).

Africa and South-east Asia are the regions where the burden of malnutrition and infectious diseases meet, resulting in a vicious cycle (8). Malnutrition results in alterations in immune function resulting in children being more susceptible to infectious diseases (65,66). Conversely, infectious diseases result in an alteration in the biological functions increasing the risk of childhood malnutrition (67). Both these disease entities occur on the backdrop of a contributing social milieu of poverty and food insecurity (13,52).

Preventative strategies directed against decreasing the incidence of paediatric HIV and malnutrition have resulted in decreasing these burdens and need to be strengthened to result in further reductions (2). However, paediatric malnutrition and HIV remain as problems faced by health-care workers in LAMIC. In a review of childhood in-patient mortality in South Africa, despite reductions in the mortality of hospitalized children, malnutrition and HIV remain major contributing factors (68). The over-arching aim of this thesis and the papers presented there-in, is to provide evidence to support the development of guidelines for the management of HIV-infected severely malnourished children. Further, to identify areas of future research for improving the clinical management of these children.

7.2 Key aspects of the main findings

This randomised controlled clinical trial comparing the treatment outcomes of severely malnourished HIV-infected children initiating antiretroviral treatment (ART) either during the acute phase of malnutrition (early arm) or following nutritional recovery (delayed arm) supported the null hypothesis. At 48 week there was no significant difference by trial arm, related to the key treatment outcomes (mortality and immunologic, virologic and anthropometric responses) as identified in the primary and secondary **objective**.

7.2.1 Key findings at baseline and during admission

In this cohort of severely malnourished HIV-infected children blood cultures performed on admission had a relatively low yield despite laboratory evidence suggesting the presence of infection. This was postulated to be most likely due to pre-hospital administration of broad-

spectrum antibiotics. Urine cultures had the highest bacterial–culture yield. TB was also a common clinical and culture-confirmed diagnosis. Induced sputum proved to have a higher yield as compared to gastric aspirates for culture-confirmed TB. None of the routine laboratory parameters performed predicted culture-confirmed TB cases. High rates of hospital-acquired infections (HAIs), especially with extended spectrum beta-lactamase (ESBL) producing Gram-negative bacteria were described.

Severely malnourished children had higher levels of microbial translocation, T cell activation and exhaustion compared to the non-malnourished children.

LPV pharmacokinetics was highly variable with lower bioavailability compared to previous studies conducted in non-malnourished and moderately malnourished children. This variability was not affected by the timing of ART initiation or TB co-treatment with “super boosted” Lopinavir/ritonavir (LPV/rtv). Fat-free mass (FFM) was found to be the only factor that affected the variability of LPV

7.2.2 Key findings during longitudinal follow-up

Using a multiple mixed effects linear regression model, the change in VL, height for age Z-score (HAZ) and weight for age Z-score (WAZ) favored the delayed arm. Most deaths occurred in the first 3 months following enrollment. Although Immune reconstitution inflammatory syndrome (IRIS) was common, it did not result in death or ART interruption. The most common IRIS phenomenon was BCG IRIS, followed by dermatological IRIS conditions. Adverse drug reactions were uncommon, and again did not result in death or ART interruption.

7.2.3 Key outcomes at week 48

Delaying ART initiation to after 14 days from admission and following nutritional improvement did not result in a statistically significant difference in immune, virologic and anthropometric responses, nor in mortality compared to early ART initiation even after adjusting for age, gender, WHO immune stage, TB and baseline laboratory parameters. Treatment success (Viral load (VL) <1000 copies/ml) or treatment failure (death or VL >1000 copies/ml) was found to be not influenced by lopinavir (LPV) pharmacokinetics (LPV exposures and levels below the limits of quantification) performed during the first 14 days following ART initiation. Patients with a bacterial pathogen isolated during admission had a trend towards higher mortality at 48 weeks. Despite 12 months on ART, levels of microbial translocation, immune activation and exhaustion remained persistently high. Immune recovery was independently affected by malnutrition, age, microbial translocation, monocyte and CD8 T cell activation.

7.2.4 Strengths and weaknesses

As a randomized controlled trial, many of the potential confounding factors were equally distributed between the two arms as seen in the baseline characteristics (chapter 2, table 8).

The study was powered to detect a mean difference of 0.5 WAZ score following nutritional recovery between the two study arms. Both arms of the study had a significant improvement in the WAZ by 48 weeks, thus with the study sample size the difference observed between the study arms was less than that projected and did not reach statistical significance. However it could be argued that a smaller difference even if statistically significant would not have been clinically relevant and alter clinical practice.

The loss to follow up (LTFU) potentially may have affected the ability of the study to detect a statistically significant difference in the primary outcome. Although every effort was made to keep in contact with parents and caregivers, in this vulnerable peri-urban population movement of patients due to economic and social reasons was a major factor determining LTFU. The sample size calculation did underestimate the numbers of patients lost to follow up. The study did identify a non-significant difference in LTFU between the arms, which is a potential area requiring a qualitative assessment of the impact of ART timing on parent/caregiver understanding and acceptance of treatment plans and rates of LTFU.

In the pharmacokinetic evaluation, as per the study protocol, adherence to LPV during hospitalization on non-assessment days was determined by in-patient prescription chart review. In the evaluation of LPV pharmacokinetics, the LPV exposures were found to be lower during the non-assessment days. The general narrative following discussion with nursing staff at the hospital was that although LPV/rtv was administered, it was difficult for staff to determine the volume actually ingested due to the small volumes and the poor palatability

7.2.5 Generalizability

The study population is generalizable to other peri-urban populations in Africa, with overall high rates of poverty, food insecurity and burden of infectious diseases. The study site is not in a malaria endemic region and therefore the influence of malaria on the mortality and anthropometric outcomes cannot be predicted.

Majority of the study population was of black African ethnic decent and HIV subtype C predominates in KwaZulu-Natal . The generalizability of the study findings to other ethnic groups and regions with other HIV subtypes requires further evaluation.

7.3 Discussion

The results presented in this thesis have both added to the existing knowledge and provided new insights into the ART management of severely malnourished HIV-infected children.

The primary research question was to determine the optimal timing of ART initiation in severely malnourished HIV-infected children. Two studies were identified in the literature

review (excluding the unpublished study due to inability to evaluate the study findings) addressing this research question.

The effect of ART timing on growth responses was assessed by Kim et al when comparing ART initiation to no ART initiation within 21 days post admission to a nutrition program (23). The study demonstrated improved growth rates, WHZ scores at 24 weeks and reduced time to nutritional recovery in children who had initiated on ART within 21 days, However in the comparator cohort only 52% (44/85) of the patients had initiated ART and may account for the relatively poorer growth responses (WHZ -0.46 +/-1.4) in the comparator cohort at 24 weeks. In results described in chapter 2, whilst there was no difference in anthropometric response (WHZ scores) between the early and delayed arms at 48 weeks, the growth rates (change in WAZ and HAZ) favored the delayed arm. Overall, while children in both arms eventually reached similar WHZ score at week 48, the rate of gain was greater in the delayed arm. Therefore there is benefit in delaying ART initiation until 14 days following the start of nutritional rehabilitation. A similar effect was seen with virologic responses at 48 weeks and the change in VL.

In the study by Njuguna I.N et al (only 32% of patients were classified as malnourished) the effect of urgent ART (mean time to ART initiation=1 day, IQR 1.1) versus post-stabilization ART (mean time to ART initiation=8 days, IQR 7.11) had no effect on mortality at 24 weeks (25). A similar effect on mortality was reported by Kim et.al (23). Interestingly in the study by Kim et al, the findings are similar to the lack of effect ART timing has on mortality as described in chapter 2. From the current evidence, ART timing has no impact on mortality at 24 or 48 weeks.

LPV pharmacokinetics was not affected by the timing of ART initiation; however it was influenced by FFM. FFM is a measure of body composition, which is made up of the lean body mass (LBM), bone mass, solid organs and water. In severely malnourished children, especially if associated with stunting, much of the initial weight gain following nutritional recovery is due to an increase in the fat mass with less LBM gained (69). Delaying ART initiation for an improvement in the FFM, would likely result in unacceptable delays in ART initiation.

There are also several practical considerations in routine clinical practice that influence timing of ART initiation. Delays in pre-test counseling of parents/caregivers, delays in getting HIV diagnostic test results especially for a young child requiring an HIV DNA PCR and delays in performing post-test counseling, inherently delay ART initiation of in-hospital patients. Difficulties in administering medication to children is of particular concern, esp when the formulations have poor palatability, require more frequent dosing and are not formulated as fix dose combinations.

Recommendation

HIV-infected children admitted with SAM can safely initiate ART after 14 days of nutritional rehabilitation

LPV pharmacokinetics has been well described in non-malnourished children (37,70–72). In a cohort of Ugandan children (48% of whom were malnourished) lower LPV exposures were described (30). In contrast, the pharmacokinetics of LPV (Cl and AUC) in this cohort of severely malnourished children described in chapter 3, was far lower than previously described. Further, delaying ART initiation to after 14 days of nutritional rehabilitation and evidence of nutritional recovery did not alter the LPV variability. Therefore even if ART initiation is delayed, therapeutic drug monitoring (TDM) and/or dose adjustment is required to achieve adequate LPV exposures. Dose adjustment of antiretroviral drugs is part of standard pharmacology practice in patients with hepatic and renal dysfunction (73) due to alterations in normal physiology. Severely malnourished children have similar physiological alterations therefore the use of TDM and dose adjustment requires further evaluation.

Recommendation

Therapeutic drug monitoring and/or dose adjustments of LPV/rtv should be further evaluated in severely malnourished HIV-infected children

Evaluation of “super-boosted” LPV/rtv in non-malnourished TB co-infected children has been previously studied (72,74). In this cohort of severely malnourished children, “super-boosted” LPV/rtv did not affect LPV variability; however, it did result in lower LPV exposures. Therefore TDM and dose adjustment should be further evaluated.

Recommendation

“Super-boosted” LPV/rtv in TB co-infected children severely malnourished HIV-infected children is supported, however TDM and dose adjustment should be further evaluated

Severely malnourished HIV-infected children are susceptible to HAIs particularly gram-negative organisms. Often these patients are nursed in general paediatric wards. There are no current Infectious Prevention and Control (IPC) policies that specifically speak to additional measures in severely malnourished children. IPC policies used in other high-risk category patients to prevent HAIs should be further evaluated.

Induced sputum rather than gastric aspirate was more likely to be culture positive for TB. The advantage of sputum induction is that it can be conducted as an outpatient and does not require a nasogastric tube insertion (75,76).

In clinical teaching, we often cite thrombocytosis, white blood cell monocytosis and high globulin fraction as supporting evidence of TB based on historical/anecdotal experience (77–

79). In this cohort of patients these were found not to have a strong prediction of culture-confirmed TB. HIV and other infections may influence these traditional makers of TB infection in children. There is a need for further research evaluating other biomarkers and TB diagnostic tests including urinary LAM (80) in these children. Children with SAM have a high burden of TB, either as a precipitating event or due to increased susceptibility to TB infection. Early recognition and treatment of adult patients with TB together with evaluation and prophylaxis in exposed children should be a priority.

The long-term impact of microbial translocation, immune activation and exhaustion in malnourished children needs further evaluation. Adult studies have linked these factors with cardiovascular risk (40).

Clinical Recommendations

Severely malnourished HIV-infected children are at high-risk of HAIs and require additional infection prevention and control practices

Induced sputum should be used for TB specimen collection

Future Research Gaps

Health systems and Social determinates resulting in delayed access to ART care
TDM and dose adjustments of LPV during nutritional rehabilitation
INH prophylaxis in children with SAM initiating ART
Effect of prebiotics or probiotics on bacterial translocation and immune activation

7.4 Conclusion

This thesis has added to current knowledge and has provided new in-sights into the management of severely malnourished HIV-infected children. Delaying ART initiation to at least 14 days after starting nutritional support was associated with improved rates of clinical improvement (changes in WAZ and HAZ) and decrease in viral load. However this delay did not improve LPV exposures. Therapeutic drug monitoring and dose adjustment of LPV during nutritional recovery should be further evaluated. These results can be used to inform changes in clinical practice and national and international guidelines for the management of severely malnourished HIV-infected children.

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APPENDICES

APPENDIX 1: GUIDELINES FOR THE INPATIENT TREATMENT OF SEVERELY MALNOURISHED CHILDREN

Ashworth A, Khanum S, Jackson A, Scholfield C. Guidelines for the inpatient treatment of severely malnourished children. World Health Organisation, 2003. Available on: http://www.who.int/nutrition/publications/guide_inpatient_text.pdf [Accessed on 16 November 2016].

Guidelines for the inpatient treatment of severely malnourished children

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Guidelines for the inpatient treatment of severely malnourished children

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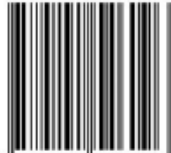
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APPENDIX 2: KING EDWARD VIII HOSPITAL PEM PROTOCOL

KEH PEM PROTOCOL PROTOCOL 1											
SIMILAC ALIMENTUM FOR PEDIATRICS 0 - 12 MONTHS											
DESCRIPTION OF FEED: A nutritionally complete hypoallergenic infant formula that is lactose free											
CONTENT OF FEED PER 100ML											
			KCAL	KILOJOULES	PROTEIN	CARBOHYDRATE	FAT				
			67	280	1.5	7g	3.75g				
STEP 1 Day 1 - 2			STEP 2 Day 3 - 4				STEP 3 Day 5 - 6				
Similac Alimentum			Similac Alimentum				Similac Alimentum				
kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg
37	1	56	56	1.5	84	74	2	111	74	2	111
Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs	Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs
2	15ml	120ml	2	20ml	160 ml	2	30ml	240ml	2	30ml	240ml
3	20ml	160ml	3	30ml	250 ml	3	45ml	360 ml	3	45ml	360 ml
4	30ml	240ml	4	40ml	320 ml	4	55ml	440 ml	4	55ml	440 ml
5	35ml	278ml	5	50ml	400 ml	5	70ml	556 ml	5	70ml	556 ml
6	40ml	320ml	6	65ml	520 ml	6	85ml	680 ml	6	85ml	680 ml
7	50ml	400ml	7	75ml	600ml	7	100ml	800ml	7	100ml	800ml
8	55ml	440 ml	8	85ml	680ml	8	110ml	880ml	8	110ml	880ml
9	65ml	520ml	9	95ml	760ml	9	125ml	1000ml	9	125ml	1000ml
10	70ml	556ml	10	105ml	840ml	10	140ml	1120ml	10	140ml	1120ml
STEP 4 Day 6 - 7			STEP 5 (Catch up) Day 7 +								
Similac Alimentum			Similac Alimentum								
kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg
93	2.5	139	112	3	167	112	3	167	112	3	167
Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours
2	35ml	278 ml	2	40ml	320 ml	2	40ml	320 ml	2	40ml	320 ml
3	50ml	400 ml	3	65ml	520 ml	3	65ml	520 ml	3	65ml	520 ml
4	70ml	556 ml	4	85ml	680 ml	4	85ml	680 ml	4	85ml	680 ml
5	90ml	720 ml	5	105ml	840 ml	5	105ml	840 ml	5	105ml	840 ml
6	105ml	840 ml	6	125ml	1000 ml	6	125ml	1000 ml	6	125ml	1000 ml
7	120ml	960ml	7	145ml	1160ml	7	145ml	1160ml	7	145ml	1160ml
8	140ml	1120ml	8	170ml	1360ml	8	170ml	1360ml	8	170ml	1360ml
9	155ml	1240ml	9	190ml	1520ml	9	190ml	1520ml	9	190ml	1520ml
10	175ml	1400ml	10	210ml	1680ml	10	210ml	1680ml	10	210ml	1680ml

KEH PEM PROTOCOL PROTOCOL 2											
ALFARE FOR PEDIATRICS 0 - 12 MONTHS											
DESCRIPTION OF FEED: A nutritionally complete semi elemental feed that is lactose and sucrose free											
CONTENT OF FEED PER 100ML											
			KCAL	KILOJOULES	PROTEIN	CARBOHYDRATE	FAT				
			70	293	2.1g	9	9				
STEP 1 Day 1 - 2			STEP 2 Day 3 - 4				STEP 3 Day 5 - 6				
Alfare			Alfare				Alfare				
kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg
33	1	48	50	1.5	72	67	2	95	67	2	95
Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs	Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs
2	15ml	120ml	2	20ml	160 ml	2	25ml	200 ml	2	25ml	200 ml
3	20ml	160ml	3	30ml	240 ml	3	35ml	280 ml	3	35ml	280 ml
4	25ml	190ml	4	35ml	280 ml	4	50ml	400 ml	4	50ml	400 ml
5	30ml	240ml	5	45ml	360 ml	5	60ml	480 ml	5	60ml	480 ml
6	35ml	280ml	6	55ml	440 ml	6	70ml	560 ml	6	70ml	560 ml
7	40ml	320ml	7	65ml	520ml	7	85ml	680ml	7	85ml	680ml
8	50ml	400ml	8	70ml	560ml	8	95ml	760ml	8	95ml	760ml
9	55ml	440ml	9	80ml	640ml	9	110ml	880ml	9	110ml	880ml
10	60ml	480ml	10	90ml	720ml	10	120ml	960ml	10	120ml	960ml
STEP 4 Day 6 - 7			STEP 5 (Catch up) Day 7 +								
Alfare			Similac Alimentum								
kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg
83	2.5	119	100	3	143	100	3	143	100	3	143
Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours
2	30ml	240 ml	2	35ml	280 ml	2	35ml	280 ml	2	35ml	280 ml
3	45ml	360 ml	3	55ml	440 ml	3	55ml	440 ml	3	55ml	440 ml
4	60ml	480 ml	4	70ml	560 ml	4	70ml	560 ml	4	70ml	560 ml
5	75ml	600 ml	5	90ml	720 ml	5	90ml	720 ml	5	90ml	720 ml
6	90ml	720 ml	6	110ml	880 ml	6	110ml	880 ml	6	110ml	880 ml
7	105ml	840ml	7	125ml	1000ml	7	125ml	1000ml	7	125ml	1000ml
8	120ml	960ml	8	145ml	1160ml	8	145ml	1160ml	8	145ml	1160ml
9	135ml	1080ml	9	160ml	1280ml	9	160ml	1280ml	9	160ml	1280ml
10	150ml	1200ml	10	180ml	1440ml	10	180ml	1440ml	10	180ml	1440ml

KEH PEM PROTOCOL PROTOCOL 3											
INFANTRINI FOR PEDIATRICS 0 - 18 MONTHS											
DESCRIPTION OF FEED: A nutritionally complete energy dense feed, suitable for catch up growth											
CONTENT OF FEED PER 100ML											
			KCAL	KILOJOULES	PROTEIN	CARBOHYDRATE	FAT				
			100	415	2.6g	10.3g	5.4g				
STEP 3 Day 5 - 6			STEP 4 Day 6 - 7				STEP 5 Day 7+				
Infantrini			Infantrini				Infantrini				
kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg
77	2	77	100	2.6	100	116	3	116	116	3	116
Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs	Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs
2	20 ml	160 ml	2	25ml	200 ml	2	30ml	240 ml	2	30ml	240 ml
3	30 ml	240 ml	3	40ml	320 ml	3	45ml	360 ml	3	45ml	360 ml
4	40 ml	320 ml	4	50ml	400 ml	4	60ml	480 ml	4	60ml	480 ml
5	50 ml	400 ml	5	65ml	520 ml	5	70ml	560 ml	5	70ml	560 ml
6	60 ml	480 ml	6	75ml	600 ml	6	90ml	720 ml	6	90ml	720 ml
7	70 ml	560 ml	7	90ml	720 ml	7	100ml	800 ml	7	100ml	800 ml
8	80 ml	640 ml	8	100ml	800 ml	8	115ml	920 ml	8	115ml	920 ml
9	90ml	720 ml	9	115ml	920 ml	9	130ml	1040 ml	9	130ml	1040 ml
10	95ml	760 ml	10	125ml	1000ml	10	145ml	1160 ml	10	145ml	1160 ml

KEH PEM PROTOCOL										PROTOCOL 4				
PEDIASURE FIBRE FOR PEDIATRICS > 1 YEAR														
DESCRIPTION OF FEED: A nutritionally complete lactose free feed that contains fibre														
CONTENT OF FEED PER 100ML														
STEP 1			STEP 2			STEP 3			STEP 4			STEP 5 (Catch up)		
Day 1 - 2			Day 3 - 4			Day 5 - 6			Day 6 - 7			Day 7 +		
Pediasure Fibre			Pediasure Fibre			Pediasure Fibre			Pediasure Fibre			Pediasure Fibre		
kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg
33	1	33	50	1.5	50	67	2	67	83	2.5	83	100	3	100
Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours
5	20ml	160ml	5	30ml	240 ml	5	40ml	320 ml	5	50ml	400 ml	5	65ml	520 ml
6	25ml	200ml	6	40ml	320 ml	6	50ml	400 ml	6	60ml	480 ml	6	75ml	600 ml
7	30ml	240ml	7	45ml	360 ml	7	55ml	440 ml	7	70ml	560 ml	7	90ml	720 ml
8	35ml	280ml	8	50ml	400 ml	8	65ml	520ml	8	85ml	680 ml	8	100ml	800 ml
9	38ml	304ml	9	55ml	440 ml	9	75ml	600 ml	9	95ml	760 ml	9	115ml	920 ml
10	40ml	333ml	10	60ml	480 ml	10	85ml	680ml	10	105ml	840ml	10	125ml	1000ml
11	45ml	360ml	11	70ml	560ml	11	90ml	720ml	11	115ml	920ml	11	140ml	1120ml
12	50ml	400ml	12	75ml	600ml	12	100ml	800ml	12	125ml	1000ml	12	150ml	1200ml
13	55ml	440ml	13	80ml	640ml	13	110ml	880ml	13	135ml	1080ml	13	160ml	1280ml

KEH PEM PROTOCOL										PROTOCOL 5				
PEPTAMEN JUNIOR FOR PEDIATRICS > 1 YEAR														
DESCRIPTION OF FEED: A nutritionally complete semi elemental feed														
CONTENT OF FEED PER 100ML														
STEP 1			STEP 2			STEP 3			STEP 4			STEP 5 (Catch up)		
Day 1 - 2			Day 3 - 4			Day 5 - 6			Day 6 - 7			Day 7 +		
Peptamen Junior			Peptamen Junior			Peptamen Junior			Peptamen Junior			Peptamen Junior		
kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg	kcal/kg	prot g/kg	ml/kg
33	1	33	50	1.5	50	67	2	67	83	2.5	83	100	3	100
Weight (kg)	Volume per 3hrly feed	Volume per 24 hrs	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours	Weight (kg)	Volume per 3hrly feed	Volume per 24 hours
5	20ml	160ml	5	30ml	240 ml	5	40ml	320 ml	5	50ml	400 ml	5	65ml	520 ml
6	25ml	200ml	6	40ml	320 ml	6	50ml	400 ml	6	60ml	480 ml	6	75ml	600 ml
7	30ml	240ml	7	45ml	360 ml	7	55ml	440 ml	7	70ml	560 ml	7	90ml	720 ml
8	35ml	280ml	8	50ml	400 ml	8	65ml	520ml	8	85ml	680 ml	8	100ml	800 ml
9	38ml	304ml	9	55ml	440 ml	9	75ml	600 ml	9	95ml	760 ml	9	115ml	920 ml
10	40ml	333ml	10	60ml	480 ml	10	85ml	680ml	10	105ml	840ml	10	125ml	1000ml
11	45ml	360ml	11	70ml	560ml	11	90ml	720ml	11	115ml	920ml	11	140ml	1120ml
12	50ml	400ml	12	75ml	600ml	12	100ml	800ml	12	125ml	1000ml	12	150ml	1200ml
13	55ml	440ml	13	80ml	640ml	13	110ml	880ml	13	135ml	1080ml	13	160ml	1280ml

APPENDIX 3: ANTIRETROVIRAL DRUG DOSING CHART FOR CHILDREN 2013



ANTIRETROVIRAL DRUG DOSING CHART FOR CHILDREN 2013

Compiled by the Child and Adolescent Committee of the SA HIV Clinicians Society in collaboration with the Department of Health



	Abacavir (ABC)	Lamivudine (3TC)	Efavirenz (EFV)	Lopinavir/Ritonavir (LPV/r)rtv	Ritonavir boosting (RTV)	Stavudine (d4T)	Didanosine (ddI)	Nevirapine (NVP)	Zidovudine (AZT)		
Target Dose	8mg/kg TWICE daily OR ≥ 10 kg: 15mg/kg ONCE daily	4mg/kg TWICE daily OR ≥ 10 kg: 8mg/kg ONCE daily	By weight band	300/75mg/m ² /dose LPV/r ^{rtv} TWICE daily	<u>ONLY as booster for LPV/r</u> (to when on RTV) TWICE daily (0.75x PV dose bd)	1mg/kg/dose TWICE daily	180-240mg/m ² /dose ONCE daily	150-200 mg/m ² /dose TWICE daily (after once daily lead-in x 2 wks)	180-240mg/m ² /dose TWICE daily	Target Dose	
Available Formulations	Sol. 20mg/ml Tabs 60mg (scored dispersible), 300mg (not scored), ABC/3TC 600/300mg	Sol. 10mg/ml Tabs 150mg (scored), 300mg, ABC/3TC 600/300mg	Caps 50,200mg Tabs 50,200, 600mg (not scored)	Sol. 80/20mg/ml Adult Tabs 200/50mg, Paeds Tabs 100/25mg	Sol. 80mg/ml	Sol. 1mg/ml Caps 15,20,30mg	Tabs 25,50,100mg (dispersible in 30ml water) Caps 250mg EC	Sol. 10mg/ml Tabs 200mg (scored)	Sol. 10mg/ml Caps 100mg Tabs 300mg (not scored), AZT/3TC 300/150mg	Available Formulations	
Wt. (kg)	Currently available tablet formulations of abacavir (except 60mg), efavirenz, LPV/r^{rtv} and AZT must be swallowed whole and NOT chewed, divided or crushed									Wt. (kg)	
<3	Consult with a clinician experienced in paediatric ARV prescribing for neonates (<28 days of age) and infants weighing <3kg									<3	
3-3.9	2ml bd	2ml bd	Avoid eating when <10kg or <3 years: dosing not established	*1ml bd	1ml bd	6ml	Avoid	5ml bd	6ml bd	3-3.9	
4-4.9	3ml bd	3ml bd		*1.5ml bd	1.5ml bd	7.5mg bd: open 15mg capsule into 5ml water: give 2.5ml	100mg od: (2x50mg tabs)	5ml bd	9ml bd	4-4.9	
5-5.9										5-5.9	
6-6.9										6-6.9	
7-7.9	4ml bd	4ml bd		*1.5ml bd	1.5ml bd	10mg bd: open 20mg capsule into 5ml water: give 2.5ml	125mg od: (1x100mg + 1x25mg tabs)	8ml bd	9ml bd	7-7.9	
8-8.9			8-8.9								
9-9.9	Choose only one option: 6ml bd OR 2x60mg tabs bd		Choose only one option: 6ml bd 12ml od		200mg nocte (1x200mg cap/tab)	2ml bd	15mg bd: open 15mg capsule into 5ml water	150mg od: (1x100mg + 1x50mg tabs)	10ml bd	1 cap bd OR 12ml bd	9-9.9
10-10.9	Choose only one option: 6ml bd OR 2x60mg tabs bd		Choose only one option: 6ml bd 12ml od		200mg nocte (1x200mg cap/tab)	2ml bd	15mg bd: open 15mg capsule into 5ml water	150mg od: (1x100mg + 1x50mg tabs)	10ml bd	1 cap bd OR 12ml bd	10-10.9
11-13.9	Choose only one option: 6ml bd OR 2x60mg tabs bd		Choose only one option: 6ml bd 12ml od		200mg nocte (1x200mg cap/tab)	2ml bd	15mg bd: open 15mg capsule into 5ml water	150mg od: (1x100mg + 1x50mg tabs)	10ml bd	1 cap bd OR 12ml bd	11-13.9
14-16.9	8ml bd OR 2x60mg tabs bd	5x60mg tabs od OR 1x300mg tabs od OR 15ml od	1x150mg tabs bd OR 8ml bd	1x150mg tabs od OR 15ml od	300mg nocte: 200mg cap/tab + 2x50mg cap/tab	2ml bd	20mg bd: open 20mg capsule into 5ml water (if the child is unable to swallow a capsule)	175mg od: (1x100mg + 1x25mg)	1 tab am 1x tab pm OR 15ml bd	2 caps am 1 cap pm OR 15ml bd	14-16.9
17-19.9	8ml bd OR 2x60mg tabs bd	5x60mg tabs od OR 1x300mg tabs od OR 15ml od	1x150mg tabs bd OR 8ml bd	1x150mg tabs od OR 15ml od	300mg nocte: 200mg cap/tab + 2x50mg cap/tab	2ml bd	20mg bd: open 20mg capsule into 5ml water (if the child is unable to swallow a capsule)	175mg od: (1x100mg + 1x25mg)	1 tab am 1x tab pm OR 15ml bd	2 caps am 1 cap pm OR 15ml bd	17-19.9
20-22.9	10ml bd OR 3x60mg tabs bd	1x300mg tabs + 1x60mg tab od	1x150mg tabs bd OR 15ml bd	2x150mg tabs od OR 1x300mg tabs od OR 30ml od	300mg nocte: 200mg cap/tab + 2x50mg cap/tab	2.5ml bd	20mg bd: open 20mg capsule into 5ml water (if the child is unable to swallow a capsule)	200mg od: (2x100mg tabs)	1 tab am 1x tab pm OR 15ml bd	2 caps bd OR 20ml bd	20-22.9
23-24.9	10ml bd OR 3x60mg tabs bd	1x300mg tabs + 2x60mg tabs od	1x150mg tabs bd OR 15ml bd	2x150mg tabs od OR 1x300mg tabs od OR 30ml od	300mg nocte: 200mg cap/tab + 2x50mg cap/tab	2.5ml bd	20mg bd: open 20mg capsule into 5ml water (if the child is unable to swallow a capsule)	200mg od: (2x100mg tabs)	1 tab am 1x tab pm OR 15ml bd	2 caps bd OR 20ml bd	23-24.9
25-29.9	1x300mg tab bd	2x300mg tabs od OR 1xABC/3TC 600/300mg tab od	1x150mg tab bd	2x150mg tabs od OR 1x300mg tabs od OR 1xABC/3TC 600/300mg tab od	400mg nocte: (2x200mg caps/ tabs)	3ml bd	30mg bd	250mg od: (2x100mg + 1x50mg tab) OR 1x250mg EC cap od	1 tab bd	1x300mg tab bd OR 1xABC/3TC 300/150mg tab bd	25-29.9
30-34.9											30-34.9
35-39.9	1x300mg tab bd	2x300mg tabs od OR 1xABC/3TC 600/300mg tab od	1x150mg tab bd	2x150mg tabs od OR 1x300mg tabs od OR 1xABC/3TC 600/300mg tab od	400mg nocte: (2x200mg caps/ tabs)	3ml bd	30mg bd	250mg od: (2x100mg + 1x50mg tab) OR 1x250mg EC cap od	1 tab bd	1x300mg tab bd OR 1xABC/3TC 300/150mg tab bd	35-39.9
>40											>40

od = once a day
usually at night
bd = twice a day

* Avoid LPV/r^{rtv} solution in any full term infant <14 days of age and any premature infant <14 days after their due date of delivery (90 weeks post conception) or obtain expert advice.
Children 25-34.9kg may also be dosed with LPV/r^{rtv} 200/50mg adult tabs: 2 tabs am; 1 tab pm

Weight (kg)	3-4.9	5-9.9	10-13.9	14-29.9	≥ 30
Cotrimoxazole Dose	2.5ml od	5ml od	5ml od	10ml or 1 tab od	2 tabs od
Multivitamin Dose	2.5ml od	2.5ml od	5ml od	5ml od	10ml or 1 tab od

PRACTICAL ADVICE ON ADMINISTRATION OF ARV DRUGS

Abacavir (ABC)

Caregivers must be warned about potential severe progressive hypersensitivity reaction which may include fever, rash, gastrointestinal & respiratory symptoms. If hypersensitivity occurs it is usually during first six weeks of therapy, symptoms tend to worsen in the hours immediately after the dose and worsen with each subsequent dose.

Caregivers or patients should discuss symptoms early with the clinician rather than terminating therapy without consultation. ABC should be stopped permanently if hypersensitivity reaction occurs. Avoid combining ABC and NVP in a regimen and avoid concurrent initiation of ABC and co-trimoxazole. Tablets (except 60mg) must not be chewed, divided or crushed; swallow whole with or without food.

Lamivudine (3TC)

Well tolerated, no food restrictions, oral solution may be stored at room temperature. Tablets are scored and can be easily divided; may be crushed and mixed with a small amount of water or food and immediately ingested.

Stavudine (d4T)

Well tolerated & palatable but oral solution requires refrigeration after reconstitution. Discard after 30 days. Capsules may be opened and powder contents dispersed in water (stable in solution for 24 hours) or mixed with a small amount of food (e.g. yoghurt). See dosing chart for further details. Consider early drug substitution if toxicity e.g. lipoatrophy develops.

Lopinavir/ritonavir (Kaletra® solution; Aluvia® tablets)

Dose is calculated on lopinavir component. Solution should be taken with food as increases absorption. Solution should be refrigerated however can be stored at room temperature up to 25°C for 6 weeks. May need techniques to increase tolerance & palatability: coat mouth with peanut butter, dull taste buds with ice, follow dose with sweet foods. Tablets must not be chewed, divided or crushed; swallow whole with or without food. Many drug interactions due to RTV inhibition of cytochrome p450.

Efavirenz (EFV)

EFV is not approved for children <3years/<10kg. Tablets must not be chewed, divided or crushed; swallow whole with or without food e.g. yoghurt or banana. Capsules may be opened and powder contents dispersed in water or mixed with a small amount of food (e.g. yoghurt) to disguise peppery taste and immediately ingested. Food, especially high-fat meals, increases absorption. Best given at bedtime to reduce CNS side-effects, especially during first 2 weeks. Consider drug-drug interactions.

Zidovudine (AZT)

No food restrictions and oral solution may be stored at room temperature. Capsules may be opened and powder contents dispersed in water or mixed with a small amount of food (e.g. yoghurt) and immediately ingested. Currently available tablets are not scored. Use with caution in children with anaemia due to potential for bone marrow suppression.

Didanosine (ddI)

At least 2 tablets of appropriate strength must be used at any one time for adequate buffering. Tablets may be chewed or crushed and dispersed in 30ml water and immediately ingested. Enteric coated (EC) capsules (250mg) are available for once daily use in children >25kg. It is recommended to administer ddi on an empty stomach at least 30 minutes before or 2 hours after meals.

Ritonavir (RTV)

Only recommended use at present is as booster for lopinavir/ritonavir when co-administered with rifampicin-containing TB treatment. Ritonavir boosting dose is not less than 0.75 x lopinavir/ritonavir dose. Should be taken with food. May be stored at room temperature, limited shelf life of 6 months. May need to use techniques described for Kaletra® to improve tolerance of bitter taste.

Nevirapine (NVP)

Once-daily dosing during the first 2 weeks of treatment reduces frequency of rash. If a mild rash occurs during the induction period, continue once daily dosing and only escalate dose to twice daily once the rash has subsided and the dose is well tolerated.

NVP should be permanently discontinued and not restarted in children who develop severe rash especially if accompanied by fever, blistering or mucosal ulceration. No food restrictions. Tablets can be crushed and mixed with a small amount of water or food and immediately ingested. Avoid NVP if rifampicin is being co-administered. Consider drug-drug interactions.

NEED HELP?

Contact the TOLL-FREE
National HIV & TB Health Care Worker
HOTLINE

0800 212 506
or **021 406 6782**

Alternatively send an SMS
or 'Please call me' to 071 840 1572

www.hivhotline.uct.ac.za



APPENDIX 4: STUDY WAS APPROVAL FROM THE POSTGRADUATE COMMITTEE OF THE UNIVERSITY OF KWAZULU-NATAL



08 June 2011

Student no: 913486811

Dr M Archary
35B Northcliffe Avenue
Westville
3629

Dear Dr Archary

Doctor of Philosophy: "Malnutrition and Antiretroviral timing in children with HIV (MATCH): A Comparison of early vs delayed initiation of HAART in severely malnourished HIV-infected children"
Department of Paediatrics and Child Health

I have pleasure in advising you that at a meeting of the Postgraduate Education Committee held on the 07 June 2011, it was recommended to the Faculty Board that you be accepted as a candidate for the above degree to be supervised by Professor R Bobat, Paediatrics and Child Health.

Enclosed please find the following:

- Guide to the procedures for Postgraduate study
- Hand Book - Nelson R Mandela School of Medicine
- Guide for presentation of Dissertation/Thesis

Please call at the Postgraduate Office at the Medical School by Friday, **01 July 2011** to finalise your registration.

Please ensure a full protocol is submitted to the Postgraduate Office within six months of registration.

I trust that your research will be both stimulating and productive, and wish you success in this venture.

Yours sincerely

Professor SJ Botha
Chair Postgraduate Education Committee

CC: Professor R Bobat
Dr R Thejpal

Studies may not begin without Postgraduate and Ethics approval.
A research application form is accessible on the UKZN Website. Completed forms are to be submitted to Postgraduate Education Administration.

Postgraduate Education Administration
Medical School Campus

Postal Address: Private Bag 7, Congella, 4013, South Africa

Telephone: +27 (0) 31 260 4327 Facsimile: +27 (0)31 260 4401 Email: heslopd@ukzn.ac.za Website: www.ukzn.ac.za

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UNIVERSITY OF KWAZULU NATAL
COLLEGE OF HEALTH SCIENCES
NELSON R MANDELA SCHOOL OF MEDICINE

MEMORANDUM

TO: Professor R Bobat Department of Paediatrics and Child Health Nelson R Mandela School of Medicine	FROM: Professor SJ Botha Chair Postgraduate Education Committee Nelson R Mandela School of Medicine 08 June 2011
--	---

Dear Professor Bobat

Doctor of Philosophy: "Malnutrition and Antiretroviral timing in children with HIV (MATCH): A Comparison of early vs delayed initiation of HAART in severely malnourished HIV-infected children"
Department of Paediatrics and Child Health
Archary M, 913486811

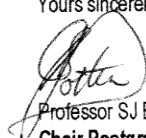
At a meeting of the Postgraduate Education Committee held on 07 June 2011 it was recommended to the Faculty Board that you be appointed as supervisor for the above candidate's studies.

Enclosed please find the following:

- Guide for procedures for Postgraduate study
- Faculty Hand Book

Please ensure a full protocol is submitted to the Postgraduate Office within six months of registration. Research application forms will be e mailed in due course.

Yours sincerely



Professor SJ Botha
Chair Postgraduate Education Committee

APPENDIX 5: APPROVAL FROM THE BIOMEDICAL RESEARCH AND ETHICS COMMITTEE (BREC) OF THE UNIVERSITY OF KWAZULU-NATAL – BFC 126/11



27 March 2012

Dr. M Archary
Department of Paediatrics
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Archary

PROTOCOL: Malnutrition and Antiretroviral Timing in Children with HIV (MATCH) A comparison of early vs. delayed initiation of HAART in severely malnourished HIV- infected children. REF: BFC126/11

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application.

The study was provisionally approved by a quorate meeting of BREC on 13 September 2011 pending appropriate responses to queries raised. Your responses dated 04 October 2011 to queries raised on 31 August 2011 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 27 March 2012.

The following related study documents have been reviewed and approved:

Amended Informed Consent form final info doc & consent form (3)
Amended screening form - Screening form (3)

This approval is valid for one year from **27 March 2012**. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/ResearchEthics11415.aspx>.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The following Committee members were present at the meeting that took place on 13 September 2011:

Professor Doug Wassenaar. Chair
Professor Dennis Pudifin, Medicine
Dr Tim Hardcastle, Surgery - Trauma
Professor T E Madiba, General Surgery
Professor Anna Coutsoudis, Paediatrics & Child Health
Professor Chris Rout, Department of Anaesthesia
Dr Shenuka Singh, School of Dentistry
Dr Saeeda Paruk, Psychiatry
Dr Halima Dawood, Medicine
Dr Randolph Green-Thompson
Dr Aslam Sathar, Medicine
Professor Steven Collings, Psychiatry
Mrs T Makhanya, Community representative

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely



PROFESSOR D R WASSENAAR
Chair: Biomedical Research Ethics Committee

APPENDIX 6: APPROVAL FROM THE DEPARTMENT OF HEALTH –
KING EDWARD VIII HOSPITAL



health
Department
Health
PROVINCE OF KWAZULU-NATAL

OFFICE OF THE HOSPITAL CEO
KING EDWARD VIII REGIONAL HOSPITAL
Private Bag X02, CONGELLA, 4013
Corner of Rick Turner & Sydney Road
Tel. 031-3603653/3016; Fax. 031-2081487
Email: rejoice.khuzwayo@kznhealth.gov.za
www.kznhealth.gov.za

Ref.: KE 27/11 (11/2012)
Enq.: Mrs. R. Sibiya
Research Programming

27 March 2012

Dr. M. Archary
Department of Paediatrics & Child Health
Nelson R. Mandela School of Medicine
UNIVERSITY OF KWAZULU-NATAL

Dear Dr. Archary

Protocol: "Malnutrition and Antiretroviral Timing in Children with HIV (MATCH), a comparison of early vs delayed initiation of HAART in severely malnourished HIV-infected children". Ref. BCF12811

Permission to conduct research at King Edward VIII Hospital is provisionally granted, pending approval by the Provincial Health Research Committee, KZN Department of Health.


Kindly note the following:-

- The research will only commence once confirmation from the Provincial Health Research Committee in the KZN Department of Health has been received.
- Signing of an indemnity form at Room 8, CEO Complex before commencement with your study.
- King Edward VIII Hospital received full acknowledgment in the study on all Publications and reports and also kindly present a copy of the publication or report on completion.

The Management of King Edward VIII Hospital reserves the right to terminate the permission for the study should circumstances so dictate.

Yours faithfully

SUPPORTED / NOT SUPPORTED


DR. O.S.B. BALOYI
ACTING CEO & SENIOR MEDICAL MANAGER

02/04/2012
DATE

uMnyango Wazampilo . Departement van Gesondheid

Fighting Disease, Fighting Poverty, Giving Hope

**APPENDIX 7: APPROVAL FROM THE KWAZULU-NATAL
DEPARTMENT OF HEALTH**



health

Department:
Health
PROVINCE OF KWAZULU-NATAL

Health Research & Knowledge Management sub-component
10 – 103 Natalia Building, 330 Langalibalele Street
Private Bag x9051
Pietermaritzburg
3200
Tel.: 033 – 3953189
Fax.: 033 – 394 3782
Email.: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

Reference : HRKM48/12
Enquiries : Mrs G Khumalo
Telephone : 033 – 3953189

10 April 2012

Dear Dr M Archary

Subject: Approval of a Research Proposal


1. The research proposal titled 'Malnutrition and Antiretroviral Timing in Children with HIV (MATCH)' was reviewed by the KwaZulu-Natal Department of Health.

The proposal is hereby **approved** for research to be undertaken at **King Edward VIII Hospital**. The study can only be conducted at Clairwood Hospital once the hospital manager has granted support for the study.

2. You are requested to take note of the following:
 - a. Make the necessary arrangement with the identified facility before commencing with your research project.
 - b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.
3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mrs G Khumalo on 033-3953189.

Yours Sincerely


Dr E Lutge

Chairperson, Health Research Committee
KwaZulu-Natal Department of Health

Date: 10/04/2012

uMnyango Wezempilo . Departement van Gesondheid

Fighting Disease, Fighting Poverty, Giving Hope

APPENDIX 8: INFORMED CONSENT FOR PARENTS OR LEGAL GUARDIANS OF ALL STUDY PARTICIPANTS

INFORMATION DOCUMENT

Date:

STUDY INFORMATION FOR PROSPECTIVE SUBJECTS

Study title: Malnutrition and Antiretroviral Timing in Children with HIV (MATCH)

Greetings patient & family:

We are asking for your permission to include your child in a research study on severe malnutrition in children infected with HIV. Your child was chosen because he/she is HIV positive, underweight and needs to be started on HIV medicines according to the South African HIV Treatment guidelines. Research is just the way to learn the answer to a question. In this study, we are trying to see when is the best time to give a very underweight child the medicines for HIV, which has not been answered by other research studies. In this study children will either be started on HIV medicines soon after being admitted to hospital (within 10 days) or will wait until his/her weight has improved or at most 4 weeks after admission before starting HIV medicines. The decision about which group your child will be in, will be made by chance (flip of a coin).

During the first two weeks of being in the study, your child will be admitted to King Edward VIII Hospital (KEH). During this time he/she will be treated for being underweight and any other infections he/she may have. After this stay at KEH, the doctors involved in the study will decide whether your child will stay longer at KEH, be transferred to Clairwood Hospital or be discharged to home depending on how sick your child still is. We will need to take blood from your child a total of 7 times over 48 weeks. We will also need to measure your child's height and weight. Finally we will need to use a special instrument called a caliper that pinches the skin to measure how thick it is. This measurement helps us know how healthy your child's muscles are.

Participating in this study will require a maximum of 4 follow-up clinic visits. We will try to make sure these visits happen on the same day as any other appointments you may have here at KEH. If you must come here so that we can take measurements for this study, we will provide you with R150 to cover your travel expenses.

All children who are a part of this study will be given a study identification number (SID). All blood samples and study information will only have this SID number and not your child's name on it to protect your family's privacy. Any information that may be linked to your child will be kept in locked cabinets. Only members of this study team will have access to this information. As part of this study, we will also need to store some samples of your child's blood so that we can look at them later. These blood samples will be stored in the Department of Paediatrics and Child Health Laboratory and the Doris Duke Medical Research Institute, NRMSM, UKZN.

These samples will not be labeled with your child's name, only their study identification number. No one but the members of this study team will be able to access these samples. The samples will be stored for at most 10 years.

The study may involve the following risks and/or discomforts. Any time we take blood from a patient with a needle there is the small possibility of infection or discomfort at the puncture site. However, trained doctors will perform the blood draws and so the risk of these problems is very low. Finally, there may be some discomfort during the pinching of the skin with the calipers.

Your child will receive the same treatment for his/her very low weight and the same HIV medicines as all other children admitted to King Edward VIII Hospital. Some of the side-effects of the HIV medicines are:

Lamivudine (3TC, Epivir®): Headache, Feeling tired, Dizziness, Upset stomach, Vomiting, Loose or watery stools, Numbness, tingling, and pain in the hands or feet, Decrease in the number of white blood cells that help fight infection, An increase in a substance in the blood (a type of a pancreatic enzyme) which could mean a problem with the pancreas, Increased liver function tests, which could mean liver damage

Abacavir (Ziagen): Headache, Nausea and vomiting, loose or watery stools, Hypersensitivity reaction.

Lopinavir/Ritonavir (LPV, Kaletra®) Pancreatitis (inflammation of the pancreas), which may cause death. If your child develops pancreatitis, your child may have one or more of the following: stomach pain, nausea, vomiting or abnormal pancreatic function blood tests , Abnormal bowel movements (stools), including loose or watery stools, upset stomach and stomach pain, Large increases in triglycerides and cholesterol in the blood, Liver problems and worsening liver disease, which may result in death. People with these conditions may have abnormal liver function blood tests, Feeling weak and tired, Headache, Rash (seen in children)

There may be no direct benefits to you participating in this study. However, we hope we hope this study will lead a greater understanding of malnutrition in HIV-positive children.

Participation in this research is completely voluntary. If you decide to refuse to participate or stop participating at any point, you and your family will not be denied treatment or any medical benefits. There are no consequences to withdrawing from the study. You may inform the researchers of your decision to withdraw from the study at any point in time.

Confidentiality: Every effort will be made to keep personal information private or "confidential". Unfortunately, we cannot guarantee absolute confidentiality. If required by the law, we may need to disclose some information. However, all results, if published will be kept anonymous; no information that can identify you will be published publicly.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Research Ethics Committee, Data Safety Monitoring Committee and the Medicines Control Council (where appropriate).

CONSENT TO PARTICIPATE in RESEARCH STUDY

I _____ have been informed about the study entitled “Early versus Delayed initiation of HAART in severely malnourished HIV-infected children” by _____, clinical research fellow.

I understand the purpose and procedures of the study.

I have been given an opportunity to answer questions about the study and have had answers to my satisfaction.

I declare that my child/wards participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

I have been informed about any available compensation or medical treatment if injury occurs to me as a result of study-related procedures.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher Dr. Moherndran Archary at Tel: (31) 2604355.

This study has been ethically reviewed and approved by the UKZN Biomedical research Ethics Committee (approval number BCF 126/11).

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus

Govan Mbeki Building

Private Bag X 54001

Durban

4000

KwaZulu-Natal, SOUTH AFRICA

Tel: 27 31 2604769 - Fax: 27 31 2604609

Email: BREC@ukzn.ac.za

Administrator: Ms P Ngwenya Email: ngwenyap@ukzn.ac.za

Chair: Email: Prof D R Wassenaar c/o ngwenyap@ukzn.ac.za

If you agree to participate, you will be given a signed copy of this document and the participant information sheet, which is a written summary of the research.

The research study, including the above information, has been described to me orally. I understand what my involvement in the study means and I voluntarily agree to participate. I

have been given an opportunity to ask any questions that I might have about participation in the study.

Signature of Caregiver

Date

Signature of Witness

Date

Signature of Translator

Date (Where applicable)

APPENDIX 9: TRANSLATION CERTIFICATE



UKZN Translation Certificate

This is to certify that the translation of the document entitled:

INFORMATION DOCUMENTS X 2

STUDY INFORMATION FOR PROSPECTIVE SUBJECTS

Appendix 9 Study title: Malnutrition & Antiretroviral Timing in Children (MATCH) Study

Appendix 10 Study title: Vitamin D Levels and Bone Health in Severely Malnourished HIV-infected Children Initiating HAART in Durban, South Africa

From English into Zulu has been completed to the best of the ability of our translator and is true to the meaning and wording of the English text. The translation was carried out by the following translator:

English into Zulu by Zanele Mtshweni

Simon Kemisho

Managing
Director

13 April 2012

Date