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Anthropometric status in HIV-infected children in Harare, Zimbabwe

A cross sectional study on baseline data as part of BREATHE trial

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Master's thesis in MED-3950 August 2020

Preface

I have always had an interest in children's health. Therefore, when I was introduced to the BREATHE trial by professor Trond Flægstad and dr. Evgeniya Sovershaeva, UiT, I knew this was going to be the project upon which I would base my master thesis. Through this project I was made aware of the burden of HIV-infection that affects many children and adolescents in Zimbabwe, further complicated by chronic lung disease (CLD) in some. UIT– The Arctic University of Norway is actively involved in the research of CLD in HIV infected children and adolescents in Zimbabwe. Through my supervisors at the University, I was given access to raw data collected at site in Harare. I would then write my master thesis with data from the project under the supervision of Evgeniya Sovershaeva, UIT.

Considering my interest, and previous experience through field work, with mapping and treating nutrition deficiencies amongst vulnerable children, it was therefore natural for me that the master thesis would have such an angle. To my knowledge there has not previously been performed studies on nutritional status among children and adolescents with HIV and CLD in the area. Thus, the purpose of the master thesis is mapping potential nutritional deficits among HIV positive children and adolescents with chronic lung disease (CLD), HIV positive children and adolescents without CLD, and healthy controllers in the same age group in Harare, Zimbabwe, respectively, and to assess potential risk factors associated with reduced nutritional status.

Deciding on the objectives, exclusion and inclusion criteria, covariates, data analysis and writing of the thesis was done be the author in cooperation with my supervisors. I would like to thank my primary supervisor, Dr. Evgeniya Sovershaeva, Department of Community Medicine, UIT, for aiding me with execution of this project, specifically in terms of contributing in the planning process, counseling regarding statistical analysis, proofreading and feedback. I would also like to thank my secondary supervisor, professor in pediatrics Trond Flægstad, Department of Clinical Medicine, UIT, for his contribution assigning me to the project, giving access to the dataset and proofreading the thesis. I would also like to thank professor in Biostatistics and Epidemiology, Tom Wilsgaard at Department of Community, UiT, for his contribution and guidance regarding statistical work on this project.

This project is done purely based on already collected data from the BREATHE trial, and there are no special resources required but the guidance of my supervisors. Thus, there has been no external funding of my project.

Martin Hanssen
30.08.2020

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Abstract

Background and objective: Undernutrition is a prevalent challenge in sub-Saharan Africa, also in Zimbabwe and Harare. HIV-infected subjects are especially exposed to anthropometric abnormalities, which can promote further disease progression and even increased mortality. Complications of HIV-infection may contribute to further deterioration of nutritional status. This study aims to assess the prevalence of anthropometric abnormalities in children and adolescents, and to assess relations with known associated factors.

Methods: A cross-sectional observational study was performed on children aged 6-19 years attending Harare Central Hospital from June 2016 – August 2018. Study participants included 213 HIV- infected subjects with chronic lung disease (CLD), 51 HIV-infected subjects without CLD, and 97 HIV-uninfected controls. Stunting, underweight and low BMI were defined by anthropometric indices: height-for-age (HAZ) and BMI-for-age (BAZ) in ages 6-19 years, and weight-for-age (WAZ) in ages 6-10 years. Analysis included descriptive statistics and logistic regression. Assessment of associated factors included immune status, viral levels, gender, presence of CLD and anemia.

Results: Overall, the study included 213 HIV-infected subjects with CLD, 51 HIV-infected subjects without CLD and 97 HIV-uninfected controls. The median age in each group was 15 years ([IQR]: 12-17/18 years) in both HIV-infected subgroups, and 10 years ([IQR]: 7-12years) in control group. In the control group, 4.1% were stunted, 4.2% were underweight and 1% had low BMI. Among the HIV-infected with and without CLD, 45.8% and 15.7% were stunted, 0% and 37.5% were underweight, 2% and 20.8% had low BMI, respectively. Among HIV-infected with CLD, prevalence of severe stunting was 14.2%, severe underweight was 12.5% and severely low BMI was 3.8%. Associated factors of stunting were HIV-infection, male gender, presence of CLD and shorter duration of ART. Low BMI was associated with male gender and CLD among HIV-infected subjects.

Conclusion: Prevalence of anthropometric abnormalities were low compared to reported prevalence from other studies regarding healthy controls and HIV-infected without CLD, although very high among HIV-infected children and adolescents with CLD. Nutritional habits, socio-demographic and socio-economic factors were not assessed, which are documented influencers of anthropometric status, in addition to the associated factors found in this study.

Abbreviations

aOR – adjusted odds ratio

ART – antiretroviral therapy

BAZ – BMI for age z-score

BMI – body mass index

CI – confidence interval

CLD – chronic lung disease

COPD – Chronic obstructive pulmonary disease

FAO – Food and Agriculture Organization of the United Nations

FEV1 – forced expiratory volume in 1 second

HAZ – height for age z-score

HIV – human immunodeficiency virus

MUAC - mid-upper arm circumference

OCHA – United Nations Office for the Coordination of Humanitarian Affairs

OR – (crude) odds ratio

SD - standard deviation

UNICEF – United Nations Children´s Fund

USAID – U.S. Agency for International Development

WHO – World Health Organization

X^2 – Chi-Square test coefficient

1 Background

1.1 Malnutrition

1.1.1 Definition of nutrition deficiency

WHO defines the term malnutrition as a common term for deficiencies, excesses and imbalances of energy or nutrient intake. (1) Thus, malnutrition includes nutritional abnormalities caused by both excess (overnutrition) and inadequate food intake (undernutrition), but also a lack or excess of micronutrients like vitamins and minerals. This study uses a definition of malnutrition as a descriptive term for deficit in one or more of the anthropometric indices. Nutrition deficit is defined as undernutrition, subcategorized as wasting, stunting, underweight and low BMI. The most common assessment of undernutrition is anthropometric measurements like weight and height, adjusted to age and sex.

1.1.2 Forms and causes of malnutrition

As mentioned, there are several subcategories of undernutrition. One may assume that nutritional deprivation initially causes weight deficits, over time followed by height/length deficits, and finally by reduced head circumference. All forms of malnutrition apply to the same sex.

Wasting is defined by a child weighing less than what is expected of an average child of the same height. This assumes a child weighs the same at a given height independently of age, which may be a useful tool for mapping undernutrition when age is not known. Nevertheless, weight and height are not independent on age in all settings. The weight and height of a child depends much on age in infancy and puberty. One of the qualities of this nutrition inadequacy is the high variability depending on short term factors like food access and health status in regard of infections among others.

Globally, short stature is commonly caused by nonpathogenic delayed growth or is genetically related. Stunting means that a child is shorter than what is expected of its age. This anthropometric abnormality usually develops over a longer period of time, often due to long-lasting food shortage, disease, recurrent infections, conditions during pregnancy or poor maternal health. World Health Organization has defined height-for-age as the best measurement of child growth (2), of which stunting is the negative outcome.

In adults, underweight is commonly measured using BMI-scale, but in children it is measured as low weight-for-age, meaning low weight compared to what is expected of children by the same age. A underweight child may be wasted or stunted, or present with a combination of both. Weight-for-age is unable to discriminate between relative height and body mass beyond childhood, and thus only applicable for growth monitoring until the age of 10 (3). Hereafter, the most appropriate measures of growth into adolescence are height-for-age and BMI-for-age, representing negative outcomes of stunting and thinness, respectively.

BMI-for-age has been suggested to be a more fitting measure of short-term undernutrition than weight-for-age in older children (4). Thinness (low BMI) is evaluated by BMI-for-age, meaning BMI in association to the age of the child or adolescent. BMI was first used as a diagnostic tool of obesity among adults, and later also for adolescents and children. Thinness reflects acute undernutrition, of which can result from recent food shortage most importantly (2), but also underlying disease, eating disorders, recurrent infections and socio-demographic factors (5, 6).

Nutritional status encompasses static and dynamic features. While someone is in the process of becoming wasted, their anthropometric status may be within normal range. Similarly, a malnourished person may be in the process of nutritional recovery. One may argue that the first person needs priority in treatment of the two, but the priority is opposite based on definition. Therefore, longitudinal as well as single nutritional assessment is clinically valuable.

1.1.3 Anthropometric measures used to define undernutrition

Stunting, underweight and thinness are statistically defined by height-for-age, weight for age and BMI-for-age respectively, below -2 Z-scores of the latest WHO growth references from 2007 (7).

1.1.4 The health effects of different forms of undernutrition

Chronic undernutrition is mainly characterized with diminished height, but also insufficient weight gain, lean body mass and adipose tissue (8). Early childhood stunting is irreversible, but may be alleviated by improved diet. It is a sign of deprivation, and carries an increased risk of morbidity. Unfortunately, children with chronic undernutrition are also exposed to impaired

neurological function and development (9, 10), and subsequently may impact their later adulthood.

Acute malnutrition affects many organ systems down to cellular level, and is therefore associated with a wide range of morbidity. Cardiovascular complications like cardiac heart failure, hypertension and tissue hypoperfusion are not uncommon. Other complications are immune dysfunction predisposing infections, imbalanced endocrine function, atrophied skin, pancreatic insufficiency, reduced microbiome development, intestinal atrophy and dysfunction resulting in reduced nutrient absorption and increased risk of bacteremia and sepsis, reduced liver and kidney function causing low metabolism, high risk of hypoalbuminemia and urinary tract infections (8). Thinness, as a type of acute malnutrition, is associated with delayed puberty maturation, decreased bone density (11), decreased cognitive function and even reduced immune function (12).

Severe acute malnutrition (SAM) presents by the syndromes marasmus and kwashiorkor, or a combination of both. Marasmus is a type of wasting syndrome, with low weight-to-height and low mid-upper arm circumference (MUAC) as a consequence of loss of muscle and adipose tissue. It is the most common form of protein-energy malnutrition, and is a result of low total calorie intake. Kwashiorkor is characterized by edema, which generally is a sign of severe malnutrition in any case. In kwashiorkor the edema begins peripherally moving cranially with severity and time. The most severe cases have generalized edema. Kwashiorkor causes reduced muscle mass, but preserved or excess adipose tissue.

Undernutrition is associated with increased morbidity and mortality. Up to 45 % of all deaths of children under the age of five years are related to undernutrition (13, 14). Over the time deaths due to nutritional conditions have decreased, much as a result of lower death rates due to HIV/AIDS among others (14).

1.1.5 Epidemiology of malnutrition

A report published in 2020 from UNICEF, World Health Organization and The World Bank Group states that the prevalence of stunting among children under five years has declined the last decade on average since the year of 2000 (15). The estimates from 2019 showed that 144 million children were stunted, down from 199,5 million in 2000. The prevalence dropped globally from 32,4 % to 21,3 % in that time period, which represents a decline of around 34 %. Africa is the only region where the number of stunted children has risen in the given time period. Also, Africa had up to 10 % higher prevalence of stunting in year 2000 compared to global levels. The stunted children in Africa then accounted for 25% of all global cases, increasing to 40% in 2019. The same year the prevalence of stunting in sub-Saharan Africa was almost 33%, down from 43% in year 2000. Comparably, the prevalence of stunting in Zimbabwe decreased from almost 34% in 1999 to 23,5 % in 2019 (16), an improvement of 30,5% over two decades.

Underweight among children under five years of age in Zimbabwe is substantially less prevalent than stunting, with reported 9,65 % in 2019, down from almost 14 % in the peak year of 2005-2006 (17). The latest national estimates of thinness among children and adolescents aged 5-19 is from 2016 when the prevalence of thinness was 5,6 %, which is an improvement from 9 % in 2006 (18). The prevalence of wasting among children under 5 years in sub-Saharan Africa was 6,8 % in 2019, of which 1,8 % was severe wasting (15). In Zimbabwe, the prevalence was 2,87 % , substantially lower than in 2005-06 when prevalence had a peak of 7,22 %. Around this time Zimbabwe was and had been struggling with political and economic unrest for a while, which may be a plausible reason for increase in prevalence of wasting and stunting.

The European Union, FAO, OCHA, UNICEF, USAID and World Food Programme collaborates on a common initiative called Global Network Against Food Crises. Annually, a report is published on the food crises of the previous year. The report of 2017 tells us about a severe drought caused by El-Niño in 2015-2016 in Zimbabwe (19). This led to a need of emergency food assistance, of which lasted until mid 2017 when the harvest improved, and thus also did the food crisis. The country´s economy is heavily based on agriculture. This makes the country vulnerable to climate disasters that affect agriculture like droughts, floods and cyclones. Although harvesting had improved during 2017, the devastating consequences of El-Niño left

the country in a poor national economic situation in the following year of 2018 (20). High inflation and high food prices were the main causes of food insecurity in Zimbabwe that year.

According to the report the burden of acute malnutrition is worse in areas affected by drought and flood. In 2019 the report states that Zimbabwe was hit by severe drought and a devastating cyclone (21). The report also states that Zimbabwe suffered from severe economic crisis. Combined with high dependency of food import and low foreign currency, that caused the worst food crisis of the last 10 years. The report estimates that the situation will continue to worsen as the COVID-19 pandemic spreads.

Zimbabwe Multiple Indicator Cluster Survey (MICS) of 2019 is a national health report that states 1 in 4 children aged under 5 years are stunted and at risk of impaired physical and cognitive growth (22). It also says that the prevalence is higher in rural areas, and lower in urban areas, of which the lowest prevalence was in Harare (19 %). The abovementioned data on stunting in this section were collected from children under the age of 5 years, and are therefore not directly comparable to my research findings. Nevertheless they provide an indication of the regional and global trends.

1.2 HIV infection

1.2.1 Epidemiology of HIV-infection in children in sub-Saharan Africa

Human immunodeficiency virus (HIV) infection is a global health problem, with the highest prevalence in sub-Saharan Africa. The global overall prevalence of HIV in 2017 was 0,5 % (23). Comparably, it was 2,6 % in sub-Saharan Africa and 9,2 % in Zimbabwe. The overall prevalence of HIV has been increasing over the past years, most likely because of increased survival as we can see from lower HIV-related death rates the last decade (23). Subsequently, the incidence has decreased over the same time period as a likely result of decreased transmission due to improved treatment coverage.

According to data published in 2017 around 70 % of all HIV-infected individuals resides in sub-Saharan Africa, equivalent to 25,7 of in total 36,9 million (24). Among these around 1,8 million are children (<15 years), and up to 90 % of them resides in sub-Saharan Africa (25). Zimbabwe is one of the African countries with the highest percentage of HIV-infected patients receiving ART (26). In 2018 the estimated number of children (0-14 years) with HIV were 84 000 in Zimbabwe, of which the estimated ART coverage for this age group was 76 % (27). UNAIDS have a global goal of 90 % treatment coverage for HIV-infected individuals.

HIV is a leading cause of both morbidity and mortality in sub-Saharan Africa. UNAIDS and WHO stated AIDS-related mortality declined by 33 % globally from 2010 to 2019 (28, 29), and by 40 % in the African Region. Studies show that ART has positive clinical effect on morbidity and mortality (30-34). ART results in suppressed HIV RNA, which leads to improved cell immunity (35). Subsequently it is a likely contributor to these promising trends.

One study from Malawi in sub-Saharan Africa showed that the effect of ART could decrease mortality by 50 % in a 10 year follow-up of HIV-infected children under five years of age (36). Similar findings have been done from studies in Europe and United States (37). Here one have found evidence that worse initial prognosis show higher reduction in mortality on ART. The Malawi study pointed out factors like poor nutritional status and weight loss as some of the most prominent independent contributors to mortality. To my knowledge, there has been little documentation of effect of ART on mortality in children, with the exception of a study from Italy that has shown promising results (38).

1.2.2 Malnutrition in HIV-infected children

Epidemiology

Malnutrition is common among African children compared to other regions of the world; 40 % of all stunted, 27 % of all wasted and 24 % of all overweight children under 5 years live in Africa (15). Although those are scary numbers, unfortunately malnutrition is especially common in HIV-infected children compared to healthy controls. The prevalence of malnutrition in HIV-infected children and adults is up to around 40 % (39, 40). Studies from Africa have found that of the ART-treated HIV-infected children, up to 16 % are wasted, 37% are stunted and 22 % were underweight (39, 41). This study found that underlying nutritional problems and repeated opportunistic infections were plausible reasons why stunting was the most prevalent type of malnourishment among HIV-infected children.

Etiology

The etiology of malnutrition in HIV-infected children is multifactorial. Studies suggest common causes being socio-economic factors, altered metabolism, opportunistic infections and low calorie intake among others (42-44). HIV infection also directly affect the gastrointestinal lumen (45), decreasing the mucosal function and leading to nutrient malabsorption. The effect of HIV-infection on intestinal dysfunction has been supported by restored luminal function and improved biochemical markers of HIV-infection after initiation of ART (46). Although, not all agree there is any association between poor growth and factors like gastrointestinal infections and malabsorption (47).

Energy requirements

Most studies in adult HIV-infected patients suggests energy requirements can increase 10 % to 30 % (48-52), increasing by severity of HIV disease, especially secondary infections. World Health Organization suggests improved diet may mediate the effect of antiretroviral therapy in adults. It is also estimated that some ARTs affect metabolism of important nutrients, making it important for HIV-infected people to maintain a healthy, nutritious diet (48).

There has to my knowledge not been registered differences in resting energy expenditure between HIV-infected children and healthy controls (47, 53, 54), with exception of opportunistic infections being triggers of increased energy expenditure. Plausible causes of the difference between adults and children may be nutritional status, dietary intake and disease

severity. Energy shortage may affect children more severely than adults due to increased energy requirements for growth in children and during their recovery of opportunistic infections (55). Subsequently, a child´s energy intake must increase accordingly to maintain normal growth.

When assessing energy requirements of an HIV-infected individual, studies mostly mention resting energy expenditure (REE) and total energy expenditure (TEE). They say an individual with HIV infection experience increased REE (42, 56), and one could subsequently think this would require increased demand of caloric intake. On the other hand, these studies also consider the total energy expenditure, which often stays normal or even decreases as a consequence of fatigue and lethargy as symptoms of HIV infection. They conclude that weight loss in this setting is caused by reduced caloric intake rather than increased energy requirements. Weight loss happens because reduced energy intake exceeds the reduction of total energy expenditure. These studies do not include children or adolescents, nor have I been able to find other studies that do.

Malnutrition and immune function

Malnutrition and HIV-infection have a synergistic negative effect on each other, and both can deteriorate the immune system, including CD4 and CD8 T-lymphocytes (43, 49, 57). Severe malnutrition impairs the immune function, and thus can attribute to higher susceptibility of infection, which in turn can promote poorer nutritional status (12). The importance of the synergistic interaction between HIV/AIDS and nutrition in sub-Saharan Africa is caused by the high prevalence of both malnutrition and infectious diseases in this area. Malaria, tuberculosis and other communicable diseases can occur frequently or even simultaneously in HIV-infected individuals (40).

Replication of HIV virus appears to be a strong contributor to poor growth in infected children because of uncompensated increased metabolic demands (53). Subsequently HIV RNA suppression seems to be beneficial for growth (47). Poor growth can also be caused or amplified by inadequate dietary intake. For this reason, particularly vulnerable are the HIV-infected children living in areas with high prevalence of malnutrition or otherwise under conditions with restricted food supply.

Both CD4 and viral load are indicators of HIV control. High viral load indicates ongoing plasma replication of the virus, meaning participants are not taking the drugs or have HIV drug resistance mutations to the drugs they use, and therefore participants are at increased risk of disease progression. Studies have shown both CD4 t-cell count and HIV RNA viral load are significant determinants of nutritional deficiency (58, 59).

Mortality

Poor nutritional status can contribute significantly to both morbidity and mortality of HIV-infected children (60, 61), even of those on ART (62-64). Studies show that malnutrition in HIV-infected individuals contributes to high mortality rates in the first months even after treatment initiation. (60). That being said, several studies suggest ART is beneficial in improving nutritional status and growth of HIV-infected children (65-67).

1.2.3 Effects of antiretroviral therapy on growth in adolescents

ART is a group of antiretroviral drugs, usually including a protease inhibitor, which efficiently reduce HIV viral replication. Before ART, undernutrition was one of the most prominent and serious As the HIV epidemic matures and the use of antiretroviral treatment escalates, many perinatally HIV-infected children are growing up to reach adolescence. Despite ART, perinatally acquired HIV-infection predisposes to chronic complications in adolescents (68). Thus, mortality is decreasing, while morbidity is increasing. Children are no longer dying of AIDS in the extent of previous times, instead they are predisposed to acquire severe chronic complications, such as growth failure and chronic lung disease (CLD) (68-70).

Growth failure, manifested by stunting and pubertal delay, is an important feature of perinatally HIV-infected adolescents (68). Untreated, these adolescents experience lag in growth and pubertal development. Although catch-up growth may be achieved by initiation of ART, effect on growth is dependent on the age of the child when treatment was initiated. Initiation of ART in late childhood will typically only have partial effect on catch-up growth (71, 72). These studies show rapid catch-up growth in the short term, but there is a lacking evidence of long term effect on catch-up growth. Growth monitoring is used as a measure of treatment effect of ART (73, 74), and is especially important in areas with restricted access to monitoring of immune markers. Lower baseline WAZ, HAZ and WHZ can be an important predictor of faster catch-up growth in short-term, although normal growth is seen to be more difficult to achieve

(71). Weight and height growth velocity is also independent predictors of survival in HIV-infected children (75, 76), of which poor growth is a predictor of mortality and poor prognosis of HIV-infection (77, 78). Even when receiving ART, severe undernutrition may be associated with increased mortality in children with HIV-infection (79).

Some known side effects of antiretroviral drugs are nausea, diarrhea, anemia and fatigue, and thereby these drugs may affect food intake and nutrition in general (40). Given the abovementioned effects of ART on nutrition and growth, nutritional assessment and intervention is of great importance for treatment outcome and survival of the infected children (60). By doing this, one can try to improve growth in children and adolescents with risk of blunted catch-up growth and pubertal development.

1.2.4 Chronic complications in HIV infected children

Chronic lung disease in children in sub-Saharan Africa and in children with HIV

HIV-associated chronic lung disease (CLD) is one of the most common complications presented in HIV-infected youth in sub-Saharan Africa (68, 80-82). CLD is a non-specific and broad term suggesting a chronic lung impairment is present. HIV-associated CLD comprises lymphocytic interstitial pneumonia , chronic infections like tuberculosis, bronchiolitis obliterans, immune reconstitution inflammatory syndrome, bronchiectasis, malignancies, and interstitial pneumonitis (83). Clinical presentation may differ between age groups (68). Diagnostic tools for CLD are often chest X-ray, high resolution computed tomography and spirometry, as supplements to symptomatology and clinical signs.

There is a lack of studies extensively assessing the prevalence of CLD in children and adolescents. A study from Zimbabwe assessing adolescents aged 10-18 years found that 86 % met the criteria of CLD (84). Other similar studies have found a prevalence of 30 % (68). Thus, based on these limited data one may not understand the full impact of CLD in HIV-infected children and adolescents. Nevertheless, it is likely to suggest CLD is a prevalent and important complication in HIV-infection that needs proper management.

Asthma and chronic obstructive pulmonary disease (COPD) are common diseases, and latest estimates from the Global Burden of Disease report from 2017 show that each of the diseases has a global prevalence of around 4 % (23). The report states that just under 6 % (3,7 million)

of worldwide deaths were caused by COPD in 2017, while the equivalent value for asthma is just under 1 % (500,000). Most of asthma-related deaths occur in low-income or middle-income countries, although it seems to be a trend of underreporting from these areas.

Asthma is the most common chronic disease among children. It is also a very common cause of disability-adjusted life years (DALYs) among children, especially in the mid-childhood ages (5-14 years) (85). Recent evidence indicates that children with persistent asthma have impaired lung growth and are at risk of developing COPD in early adulthood (86).

In sub-Saharan Africa, as in the rest of the world, many children are affected by pulmonary disorders. The national report for Zimbabwe from the Global Burden of Disease report estimates the combined prevalence of COPD and asthma to be around 6 % among children under 14 years of age (23). Combined, they are the cause of death in 0,6 % of all cases in this age group.

Undernutrition in HIV-infected children with chronic lung disease

Malnutrition is known to exacerbate many complications and HIV-associated chronic disease such as CLD (49). Consequently malnutrition may be considered a risk factor of HIV progression (87). Moreover, undernutrition could increase the risk of developing CLD in HIV-infected children and adolescents (82). There is a lack of published studies reporting the prevalence of undernutrition among HIV-infected children with airway abnormalities in sub-Saharan Africa.

Lung function in HIV-infected subjects has been shown to benefit of early onset of ART, in which nutritional intervention may have additional effect (88). Despite the beneficial effects of ART, some studies suggest that ART may contribute to lung impairment (89, 90). Because nutritional status is associated with HIV-associated CLD, the effects of ART on nutritional status in the study population may be complex.

1.2.5 Pathogenesis and immunological classification of HIV

In the pathogenesis of HIV infection, many target cells are involved. Among these, the CD4+ T-cell is the most important. This cell is targeted by the virus and leads to CD4 T-cell depletion. The activity of the virus and hence also the immune status is assessed by measuring the amount of CD4 T-cells in our blood (per mm^3) or the percentage of these cells. This method is preferred for classification of HIV-related immunodeficiency. Both the levels of CD4+ T cells and HIV DNA level are thought to be predictors of HIV progression (91).

WHO´s classification of HIV is divided into clinical and immunological assessment (92). Clinically, HIV is divided into four stages, from asymptomatic to severely symptomatic. The immunological classification is most often preferred. Among children younger than 5 years, CD4 count tends to vary more within an individual than CD4 percentage (%CD4+). This means CD4 percentage is the preferred measurement of immunological status in children younger than 5 years. For older children than 5 years of age, both CDC classification system and the WHO classification system use CD4 count as the main assessment tool in immunological classification (92, 93). Because CD4 count tends to fluctuate within an individual depending on current illness, physiological changes or test variability, WHO claims one individual value is not by far as informative than several measurements over time. Analyzing CD4 count does not require advanced equipment, and is much more available in resource-limited settings. Nevertheless, neither of these classification systems are based on data collected from African nor Asian children, only from Europe and United States.

1.2.6 Relationship between nutrition and other clinical parameters

Anemia

Anemia is a very common condition, in 2016 just over 40 % of children under 5 years of age were anemic worldwide (94), although with a decreasing trend. The prevalence is similar in Zimbabwe at around 42%. Studies show that anemia is associated with impaired physical growth in children (95-99), where both may cause the other.

Anemia has adverse effects on the immune system, and contributes to increased morbidity from infections (96), although iron supplementations has been reported to promote immune function and thereby reduce morbidity from infectious disease. These benefits has also been reported regarding HIV-infection (100). ART has been reported to improve hemoglobin levels

in HIV-infected children, which supports the suggestion that anemia is directly related to HIV-infection (101). There is a wide range of pathophysiologic mechanisms explaining the presence of anemia with HIV-infection, including nutritional deficiencies amongst others, more specifically iron, folic acid and vitamin B12 (102). Anemia is a predictor of disease progression and mortality among HIV-infected children (102, 103).

Tuberculosis

Malnutrition is a predictor of tuberculosis (TB) and associated with worse outcome of the disease (104, 105), of which weight loss is a common clinical feature (83). Tuberculosis is also strongly associated with HIV-infection (104, 106). TB remains the leading cause of death among people living with HIV, accounting for around one in three AIDS-related deaths (29).

1.3 Justification for the study

There is no question that nutritional monitoring is of great importance in vulnerable patient groups such as people suffering from chronic lung disorders and/or HIV-infection. As stated in the aforementioned summery of research on the area, it has been shown that poor nutrition can have a negative influence of treatment and progression of these diseases, but also be a result of insufficient disease control and delayed initiation of treatment. Subsequently, the assessment of anthropometric status may have a preventive effect on these individuals if necessary interventions are initiated.

2 Objectives

This study will provide an overview of anthropometric status in children and adolescents aged 6-19 in sub-Saharan Africa. Anthropometric status will be assessed by height-for-age, weight-for-age and BMI-for-age z-scores. The objective will be to compare anthropometric status in HIV-infected and HIV-uninfected subjects. In addition, we will investigate the association between anthropometric parameters and immunological, virologic and clinical parameters in HIV-infected group.

The main objective of this study is to investigate anthropometric status and presence of anthropometrical abnormalities in HIV-infected children and adolescents with chronic lung disease (CLD).

Sub-objectives are:

1) Investigate differences in anthropometric parameters between HIV-infected with CLD, HIV-infected without CLD and a comparable group of healthy HIV-uninfected individuals.

2) Investigate associations between anthropometric abnormalities and immunological, virologic and clinical parameters in HIV-infected group.

3 Materials and methods

3.1 Study design and population

This is a cross sectional study performed using baseline data collected as part of BREATHE trial (NCT 02426112) (107). The BREATHE trial (Bronchopulmonary function in response to azithromycin treatment for chronic lung disease in HIV infected children) is an ongoing randomized controlled trial that investigates the prophylactic effects of azithromycin treatment in children and adolescents with HIV-associated chronic lung disease (CLD) in sub-Saharan Africa. The two BREATHE trial sites are located in Harare, Zimbabwe and Blantyre, Malawi. To perform my study, a group of HIV-uninfected participants were enrolled. Data for this thesis project has been collected from children attending Harare central hospital during the period June 2016 – August 2018. The author was not personally involved in data collection process. Data was collected from 361 subjects aged 6-19 years, of whom 213 are HIV-infected with HIV-associated CLD, 51 are HIV-infected subjects without CLD, and 97 are HIV-uninfected controls. These groups represent 59,0 %, 14,1 % and 26,7 % of the total population respectively.

We used the following parameters in our study:

- Spirometry results (FEV1 Z-scores)
- HIV RNA viral
- CD4 count
- Sex
- Weight
- Height
- Age
- Previous tuberculosis*
- Time on ART
- Age when ART was initiated
- Anemia

* Data only available for HIV positive subjects.

3.2 Inclusion and exclusion criteria

The inclusion criteria for the HIV-infected in this thesis were as follows:

- Participant has chronic lung disease [1]
- Home address in Harare
- Informed consent to participate in the trial
- Participant is receiving first- or second line ART ≥ 6 months
- Participant is aged 6-19 years
- Participant has perinatally acquired HIV-infection

The exclusion criteria for the HIV-infected in this thesis were as follows:

- Diagnosis of tuberculosis at enrolment
- Acute respiratory tract infection during enrolment
- Pregnancy and breastfeeding
- Participant is smoking
- Lack of understanding of the study procedure or uncooperative behavior

See BREATHE study protocol for supplementary and detailed inclusion and exclusion criteria (107).

The inclusion criteria for HIV-uninfected subjects (108) in this thesis were:

- No HIV infection
- Participants are from the same catchment area as the HIV-infected group
- Participants have same socio-demographic backgrounds
- Participant is aged 6-19 years
- Participant has no prior history of heart/lung diseases (including history of TB)
- No reported chest pain after exercise, shortness of breath during exercise or chronic cough
- Normal lung function

[1] Criteria applies to group of HIV-infected with CLD. For more information, see section 3.3.2 Spirometry.

3.3 Study procedures

At study enrollment several tests and measurements were made of the participants by research nurses and assistants. These includes anthropometric measurements, collection of nasopharyngeal and sputum samples, blood samples and spirometry. Description of the use of these data are listed in the following subsections.

3.3.1 Anthropometric measurements

Weight and height were measured by study nurses at enrolment in accordance with WHO guidelines. In this study the term "*anthropometric status"* will be used instead of the term "*nutritional status",* as it is reflected merely by the anthropometric indices we include in this study, and excludes biochemical, clinical, demographic, environmental and social factors related to nutritional status of which we lack sufficient data.

This study will assess anthropometric indices as predictors of nutritional status, including stunting, underweight and thinness. These subtypes of undernutrition are measured using z-scores, which are measures explaining how many standard deviations a sample score is from the population mean. The z-scores used in this project were created by WHO from a diverse global population to make a reference point of the normal growth curve of a child and adolescent. By using z-scores, one can assess whether a sample is above or below average (z-score = 0), reflecting over- or undernutrition respectively. Weight-for-age, height-for-age, BMI-for-age z-score <2 standard deviations represents underweight, stunting and low BMI/thinness, respectively.

The anthropometric z-scores of stunting, thinness and underweight used in the BREATHE trial were originally calculated using British growth standards. These z-scores were later recalculated by me in accordance to WHO growth references from 2007 using WHO AnthroPlus SPSS macro software.

WHO considers wasting as not applicable for children over 5 years of age, thus making reference data of wasting unavailable for the study population. Weight-for-age reference data beyond age 10 are not available because WHO argue the pubertal growth spurt may distort the accuracy or applicability of this indicator in tall children who may be mistaken for having excess weight (by weight-for-height z-score) (109). Reference data on stunting and thinness are

available for children aged 5-19 years, equivalent to 61-228 months. All anthropometric z-scores below -3 SD means the subject is either severely stunted, underweight or thin.

3.3.2 Spirometry

Chronic lung disease (CLD) was in this study defined by spirometry as forced expiratory volume in 1 second (FEV1) z-score less than -1.0 SD with no reversibility, defined as <12 % improvement in FEV1 after participant uses spacer to inhale 200 µg salbutamol, which is a type of bronchodilator. Spirometry was performed using the EasyOne™ spirometer (NDD Medical Technologies Inc., Andover, MA, USA) in accordance with the American Thoracic Society guidelines.

3.3.3 CD4 count and viral load measurements

Blood samples were used to measure CD4 count with the Pima™ Analyser (Alere, Orlando, FL, USA) and to perform viral load testing (Xpert™ HIV-1 Viral Load; Cepheid). Viral suppression was defined as <1000 copies/mL. CD4 count was used to define four levels of HIV associated immunodeficiency in accordance with WHO definitions (92); none, mild, advanced and severe immunodeficiency, defined as number of CD4+ t-cells per mm^3 >500, 350-499, 200-349 and <200, respectively. Based on these definitions, a binary variable was created dividing immune status into no to mild and advanced to severe immunodeficiency, respectively.

3.3.4 Hemoglobin

Anemia was defined using WHO´s age-adjusted recommendations for cut-off values of hemoglobin to define anemia (110). WHO defines three levels of anemia: mild, moderate and severe. This study does not discriminate between these levels, and only uses binary outcome of anemia or no anemia. The definition of anemia is age-specific, and the cutoffs are as follows: ≤114 for children aged 5-11 years, ≤ 119 for children aged 12-14 years and non-pregnant women aged > 15 years, ≤ 129 for men aged > 15 years.

3.4 Statistical analysis

Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) version 26. BMI-for-age, height-for-age and weight-for-age z-scores were calculated using WHO AnthroPlus macro for SPSS (111). The macro is a software for the global application of the WHO Reference 2007 for 5-19 years to monitor the growth of school-age children and adolescents.

Descriptive statistics were used for data reporting, including central tendency measures and frequencies for all three study groups; HIV infected with CLD, HIV infected without CLD and HIV uninfected. All variables were tested for normality with Shapiro-Wilk test and graph representation. For normally distributed variables, the variable descriptives were reported with means and standard deviations, and differences between groups were assessed using the Student t-test for continuous variables and Chi-Square test for categorical variables. If more than 20 % of cells had expected count less than 5, the Fischer´s exact test was used. Non-normally distributed continuous variables were presented by medians and interquartile range (IQR), and compared between groups by Mann-Whitney U test.

To assess sub-objective number 1, the approach was first to investigate the potential effect of HIV-infection on anthropometric status. Accordingly, prevalence of anthropometric abnormalities were compared between HIV infected subjects without CLD and HIV uninfected subjects using Chi-Square test. Then logistic regression was performed to asses any association between HIV infection and the particular anthropometric abnormality(-ies), adjusting for age, gender and chronic lung disease. In addition, logistic regression was performed to assess the association with anthropometric abnormality for other independent variables such as HIV viral load, CD4 count, CLD and anemia.

When assessing the association between independent categorical variables and anthropometric abnormalities, binary variables were constructed with two outcomes; >/< -2 standard deviations of each of the anthropometric z-scores. Crude odds ratios were obtained for each of the independent variables, as presented in tables in appendix. Adjusted odds ratios were obtained using logistic regression and adjusting for covariates.

To perform logistic regression analysis, the assumption that there is no intercorrelation between independent variables must be met. Correlation analysis showed relationship between age and CD4 count, as well as between CD4 count and viral load. T-test showed significant difference in CD4 count between genders, although there is no difference in CD4 levels as categorical variables. Continuous variables like hemoglobin, CD4 count and HIV viral load were recoded into binary categorical variables. Hemoglobin was used to define anemia. Similarly, viral load was used to identify viral suppression, and CD4 count was used to categorize immunodeficiency into two levels: no to mild immunodeficiency (CD4 count $\geq$ 350 per mm^3), and advanced to severe immunodeficiency (CD4 count <349 per mm^3). The independent variables of CD4 levels, viral levels, CLD status were only adjusted for age and gender generally, and HIV infection, viral levels and CD4 levels were additionally adjusted for CLD status. All other covariates were not adjusted for because of intercorrelation/-relationship.

Due to small sample size of subjects suffering from underweight (weight-for-age z-score <2 SD), analysis of odds ratios were not performed when assessing associations with independent variables.

3.5 Ethical approval

The study is part of The BREATHE trial, which is approved by the Medical Committee for Medical and Health Research Ethics (Northern Norway), London School of Hygiene and Tropical Medicine Ethics Committee (United Kingdom), College of Medicine Research Ethics Committee (Malawi), Medical Research Council of Zimbabwe, Harare Central Hospital Ethics Committee and by the University of Cape Town Ethics Committee.

Only patients and their caregivers who gave written assent and informed consent, respectively, to participate in the BREATHE trial were considered as potential participants of the study.

4 Results

4.1 Characteristics of study groups

An overview of demographic, anthropometric and clinical parameters of the study participants are presented in table 1. Non-normal distributions in regard of study groups were seen for most anthropometric z-scores, FEV1 z-score, HIV viral load, CD4 count and other variables like age when ART was initiated and years treated with ART. Distribution analysis is presented in table 1, and characteristics of the anthropometric abnormalities are presented in table 2.

4.1.1 Demographics

There were in total 361 study participants, of which 97 (26.9%) were HIV uninfected, 213 (59.0%) were HIV infected with CLD and 51 (14.1%) were HIV infected without CLD.

Gender distribution was equal among HIV uninfected (48.5% female, 51.5% male), and among HIV infected subjects with CLD (45.1% female, 54.9% male). Compared with the latter group, there was a significantly different gender distribution in the HIV infected without CLD (64.7% female, 35.3% male , P 0.013). The median age was 15 (12-17/-18) years among the HIV infected subjects, which was significantly higher than that of HIV uninfected subjects with a median age of 10 years (p 0.000). The age distribution was also similar between the HIV infected subgroups, with the majority being older than 12 years. There were no children older than 16 years among the HIV uninfected, with the majority being 6-9 years of age (49.5%). This age group constituted the fewest subjects in HIV infected with (11.3%) and without CLD (15-7%).

4.1.2 Anthropometric abnormalities

The most frequent form of anthropometric abnormality in almost all study groups was stunting (height-for-age z-score <-2 SD). Nevertheless, there was large discrepancy of prevalence of stunting between study groups. The prevalence was significantly higher among HIV infected with CLD (45.8%) than in HIV infected without CLD (15.7%)(p 0.000). Furthermore, the prevalence of stunting in the latter group was significantly higher than in the HIV uninfected group (4.1%, P 0.024). HIV infected subjects with CLD amounted to 89 % of all stunted individuals, although the majority (54.2%) in this group was not stunted. Chi-

Of the 97 stunted HIV infected subjects with CLD, 30 were severely stunted, comprising 14.2% of the total study group. This finding was substantially higher than in any of the remaining groups, with only 1 of HIV infected without CLD and none of healthy controls were severely stunted.

The prevalence of low BMI (BMI-for-age z-score <-2 SD) differed substantially between HIV infected with (20.8%) and without CLD (2.0%)(P 0.000). There was no difference in prevalence of low BMI between the HIV infected without CLD and HIV uninfected (P 0.656). 3.8% of HIV infected with CLD had severely low BMI-for-age z-scores. The prevalence of underweight (weight-for-age z-score <-2 SD) was 37.5% (n=9), 4.2% (2) and 0% (n=0) in the groups of HIV infected with and without CLD and HIV uninfected, respectively. The difference of prevalence between groups was not significant, probably due to small sample sizes and low frequencies. The prevalence of severe underweight was 12.5% in HIV infected with CLD, 0% among HIV infected without CLD and 2.1% in HIV uninfected. Among HIV infected with CLD and low BMI, 77.3% were males (P 0.001) and 45.5% were aged 13-16 years (table 2).

4.1.3 Clinical parameters

Groups of HIV infected subjects differed significantly regarding CD4 count. The median level of CD4 count was 565 (343-755) in the HIV positive group with CLD, and 680 (439-917) in the HIV positive group without CLD (P 0.026). Among the HIV infected subjects with CLD there were 157 (73.7%) with no or mild immunodeficiency and 56 (26.3%) with advanced immunodeficiency. When categorizing immune status into two groups defined by CD4 count ≥/< 350 per mm^3, the distribution was not significantly different between groups (P 0.372). No study subjects were suffering from severe immunodeficiency. There was also no significant difference in HIV viral load between CLD positive and CLD negative subjects, with the respective medians being 372 (46-8970) and 278 (39-2360). Nor were there any significant difference between these groups when assessing viral suppression (P 0.150). Chi-square test revealed that both viral status and immune status as binary variables are independent on sex in both HIV-infected subgroups.

The median age when ART was initiated was 8 (5-11) years in subjects with CLD and 8 (6-11) years in children without CLD (P 0.573). This similarity between groups could also be found regarding number of years treated with ART: 6.4 (3.9-8.2) years in subjects with CLD and 6.9 (4.1-8.7) years in subjects without CLD (P 0.365).

The HIV infected groups also shared similar prevalence of anemia: 30.3% of CLD-positive and 27.5% of CLD negative subjects, making them substantially more anemic than the HIV uninfected controls (9.8%, P 0.009 for CLD negative vs. HIV negative subjects).

One may notice that the median spirometry FEV1 z-score is significantly higher among HIV infected without CLD (0.54 (0.25-0.79)) than among those who were not infected with HIV (-0.24 (-0.61 to -0.32))(P 0.000). The latter group were included in the study with an inclusion criteria of normal lung function.

There was significantly higher occurrence of prior tuberculosis in those HIV-infected with CLD (33.8%) than in those without CLD (15.7%)(P 0.017). HIV uninfected were selected on inclusion criteria of no prior history of tuberculosis. Only HIV infected participants with CLD were tested for asthma, of whom only 2.8% had it.

4.2 Associations between anthropometric measurements and explanatory variables

Logistic regression was used to analyze the association of potential predictors with anthropometric status. The following parameters where tested as predictors: immune status defined by CD4 count, HIV viral suppression, CLD status and anemia, in addition to HIV-infection in regard to stunting.

Stunting

Regarding age distribution, the majority of stunted subjects with HIV-infection were older than 12 years (see table 2), although there was not any significant relation with age groups in neither of the subjects with (P 0.204) or without CLD (P 0.950). Stunting was not dependent on sex among subjects who were HIV uninfected and those HIV infected without CLD. Among stunted HIV infected with CLD, males (68%) constituted more than twice as many as that of females (32%) when performing Chi-Square test (X^2 11.94, P 0.001). Among HIV-

infected individuals, the stunted were more likely to be males (aOR 3.18, P 0.000) and have chronic lung disease (aOR 3.96, P 0.001). The likelihood of high viral load, immunodeficiency or anemia were not significantly altered among the stunted subjects with HIV-infection.

There was a significantly higher prevalence of stunting among HIV-infected subjects without CLD compared to HIV-uninfected subjects (table 1). The association between stunting and HIV-infection was significant (aOR 6.34, P 0.003). The majority of stunted in both HIV-infected subgroups had low viral loads and none to mild immunodeficiency, although the differences were not significant using Chi-Square test. The stunted HIV-infected subjects were not significantly more likely to have decreased immune status, high viral load or anemia compared to those not stunted. In addition to the predictors presented in table 3, the association of age when ART was initiated and time on ART were assessed for being stunted. The results showed HIV-infected subjects who were stunted were more likely to have received ART for a shorter period of time (aOR 0.89, P 0.038).

Thinness/low BMI

Like stunting, low BMI was independent on sex in both groups of HIV uninfected and HIV infected without CLD. Using Chi-Square test, males constituted more than three times as many than females (77.3% vs. 22.7%) among HIV infected with CLD and low BMI (X^2 11.94, P 0.001) (table 2). Low BMI was associated with male gender among the HIV-infected subjects (aOR 3.96, P 0.002) (table 5).

20.8% of HIV infected subjects with CLD, 2% of HIV infected without CLD and 1% of HIV uninfected subjects were too thin. Subsequently, 95,7 % of subjects scoring <2 SD of BMI-for-age z-score were HIV infected with CLD. Thus, the likelihood of reduced BMI were significantly increased by having CLD in addition to HIV infection (aOR 11.47, P 0.019). Among subjects with HIV infection and CLD the majority of those with low BMI had viral suppression (59.1%) and none to mild immunodeficiency (81.8%), although non-significant. Neither HIV viral load, immune status nor anemia affected odds ratio of being stunted significantly. Among those severely thin (N 9), defined as BAZ < 3 SD, there was no significant difference between any disease groups regarding HIV- and CLD-status.

5 Discussion

This study aims to describe children and adolescents with perinatally acquired HIV infection with and without chronic lung disease, and to describe comparable controls with no HIV-infection. These study participants are described in regard to anthropometric abnormalities and the associations with relevant parameters, using data collected as part of the BREATHE trial from patients attending Harare central hospital in Zimbabwe from June 2016 to August 2018.

Several important findings could be highlighted from the results of this study. The prevalence of stunting, underweight and low BMI were relatively low in HIV uninfected subjects compared to expectations based on earlier collected data from this region, 4.1%, 4.2% and 1%, respectively. The prevalence of stunting and low BMI were significantly higher among HIV infected subjects with versus without CLD (table 1). The prevalence of stunting, underweight and low BMI was 15.7%, 0% and 2% among HIV infected without CLD, and 45.8%, 37.5% and 20.8% among HIV infected with CLD, respectively. Stunting were significantly associated with HIV-infection, male gender, presence of CLD and shorter duration of ART. Low BMI was associated with male gender and CLD among HIV-infected subjects.

Stunting is of significant importance because WHO regards height-for-age the best anthropometric measurement of child growth (2), and because stunting was the most frequent anthropometric abnormality throughout all study groups but one. Stunting is a sign of chronic undernutrition, and is typically caused by long-lasting conditions like food deprivation, famines, poverty, low or no parental education and chronic diseases. In Zimbabwe, these conditions have been persistent. In the last years Zimbabwe has been heavily affected by natural disasters threatening their food supplies, which in turn has led to severe economic unrest. The prevalence of stunting was 4.1% among HIV-uninfected subjects. National estimations from Zimbabwe have reported a prevalence of stunting among children under five years of age at 27% in 2015 and around 23-25% in 2019 (16, 22). A national health report from 2019 found that prevalence was lowest in urban areas, of which the lowest prevalence was reported in Harare at 19% (22). Differences in time of data sampling, sample size and age groups may be important factors contributing to the large discrepancy between earlier reported prevalence and findings of this study among HIV-uninfected subjects. All stunted

children without HIV-infection belonged to the youngest age group of 6-9 years, which may be indicating a higher tendency of stunting in younger children, as previous research has indicated (39). Nevertheless, this association is biased by the fact that children aged 6-9 years constituted half of the study group. HIV uninfected subjects were selected with inclusion criteria of being healthy, at least in regard of diseases affecting heart and lungs, among others. This must also be considered when comparing results with prior prevalence of anthropometric abnormalities, because the general population being compared may not be as healthy as the HIV uninfected controls in this study.

15.7% of HIV infected subjects without CLD were stunted, which is also lower than that of the mentioned recent estimates of the general young population in Zimbabwe and Harare. This may be caused by gender distribution with a majority of females, in which seems to be less associated with anthropometric abnormality. Or it may also be due to a tendency of younger children being more susceptible to chronic undernutrition (39), making their estimates higher than for our older study population, despite having HIV-infection. Comparison with similar studies is difficult due to the specific circumstances of this study. A study performed in 2011 among HIV-infected individuals, of whom the majority received ART, reported a prevalence of chronic malnutrition at 24% in ages 5-19 years (39). The majority of those with chronic malnutrition were asymptomatic in regard of their HIV-infection (WHO clinical stage I) and had no immunodeficiency. The basis of comparison between the findings of this report and the results of this study is limited by two factors in particular. First, chronic malnutrition is not equivalent to stunting due to use of different/multiple anthropometric indices in definition of chronic malnutrition. Second, the sample sizes of these groups are widely different (n=1350 vs. n=51). Therefore, it is challenging to make these findings transferable to a general population of HIV infected beyond the scope of this study.

When focusing on the HIV-infected subjects with HIV-associated chronic lung disease, prevalence of stunting increased significantly compared with the other groups to 45.8% (P 0.000). The prevalence of underweight and low BMI were also markedly higher in this group, 37.5% and 20.8%, respectively. There seems to be a lack of studies assessing undernutrition in relation to ART-treated HIV-infection with presence of chronic lung disease. It has earlier been suggested a possible association between undernutrition and HIV-associated chronic lung disease (82), although I could not find studies assessing this relation clinically. Chronic

lung disease may by itself cause growth deficit and delayed development in children and infants (112, 113), although this relation is also seen reversed, as undernutrition may cause impaired lung function in children and adolescents (114, 115). The increased prevalence of all anthropometric indices in HIV-infected subjects compared to those without CLD, may therefore be attributable to an additive or synergistic effect of HIV-infection and chronic lung disease. This could also explain the high prevalence of severe undernutrition in this study group.

This study found that stunting was significantly associated with male gender, chronic lung disease, HIV-infection and shorter duration of ART. Male gender has been associated with anthropometric abnormality in several studies of HIV-infected children (39, 116-118). The benefits of ART on undernutrition has been reported numerously (119-122), of which weight gain seems to me more rapid and prominent than height gain. Unlike stunting, underweight and low BMI are caused by short term factors like diarrhea, opportunistic infections and malaria, all in which are fairly common among HIV-infected children. When ART increases immune function, the susceptibility of contracting these conditions decreases, accompanied by an opportunity of catch-up growth in weight followed by height gain.

The association with duration of ART has been reported in other studies (39). Younger age at initiation of ART is reported to have a preventive effect on undernutrition (121), as evidence suggest only partial catch-up growth can be achieved when ART is initiated in late childhood (71, 72). Nevertheless, age at initiation of ART was not significantly associated with stunting or low BMI in this study. Neither was levels of CD4 count or HIV viral load, as supported by some studies (76, 123-125). Contradictory findings have been reported (121), which may be caused by differences in study design, population, underlying disease and nutritional status.

Before WHO in 2015 published recommendations of immediate ART initiation upon diagnosis of HIV-infection in all children and adolescents (126), initiation was based on HIV clinical stage or CD4 levels. Thus, any of the study subjects diagnosed before 2015 could potentially have gone years untreated, with the subsequent disposition of developing growth deficits. Subjects in this study who initiated ART before 2015 may therefore have done so on the indication of disease severity, in which anthropometric deficits may have had time to develop beforehand. This potential effect on the findings could not be investigated without longitudinal anthropometric and clinical data.

ART has a positive impact on catch-up growth in HIV-infected children. Nevertheless, this effect is dependent on age of ART initiation, where initiation in late childhood may result in only partial catch-up growth. Thus, ART is not sufficient for reaching normal growth (71, 72, 123). Despite ART, there are still high prevalence of anthropometric abnormalities in this study, first and foremost among subjects additionally suffering from chronic lung disease. Therefore, ART alone may not be sufficient to restore an already endangered anthropometric status, and may be hindered by other contributing factors like anemia, or socio-economic and socio-demographic background (41).

The prevalence of anemia is 9.8% in the control group, and increases significantly to 27.5-30% in the HIV-infected groups. There is no significant difference in prevalence of anemia between HIV-infected subgroups based on CLD-status. Subsequently, this may indicate that the significant difference in prevalence of anemia between HIV-uninfected and -infected is due to HIV-infection alone. Given the consensus of anemia predisposing impaired growth in children, one could suggest it probable to have some effect on anthropometric status in the study groups. Nevertheless, analysis did not show any significant associations implying such.

5.1 Limitations and strengths of this study

Results of this study need to be interpreted cautiously in light of some important limitations. First, the results cannot be generalized beyond the circumstances of which this study was performed. Second, this is an observational cross-sectional study, and can therefore only describe associations, not causality, between variables and outcomes like anthropometric abnormalities.

Third, data on nutritional diet, socio-demographic or socio-economic conditions were not available. It has been reported that rural habitation, lower economic status and lower maternal education level predispose to higher prevalence of stunting among children in Zimbabwe (22). Socio-demographic factors make an impact on anthropometric status not only in Zimbabwe, it is a worldwide phenomenon (6, 127, 128). This study lacked socio-demographic data on habitational area, maternal/parental factors like educational level and health, socio-economic status and educational levels of the subjects. Thus, one could not assess the relationship between

socio-demographic and -economic factors and anthropometric status, which potentially could be of great importance.

Fourth, analysis of associations could not be performed regarding underweight due to small samples of affected participants. Weight-for-age as a measure of underweight only applies to an age interval of 5-10 years and subsequently make up a smaller part of the study population. The small sample size of underweight subjects limits the value of assessment of associated factors, and these analyses were therefore not performed. Finally, the association between HIV-infection and stunting could be biased by any influence ART may have on undernutrition. From a methodological standpoint, a better study design would be to include ART-naïve HIV-infected subjects, although this would be ethically unacceptable. However, the inclusion of an HIV-uninfected control group was a strength of this study.

6 Conclusion

Compared to the prevalence reported in similar studies from the region, anthropometric abnormality was not as frequent among HIV-uninfected controls and HIV-infected without CLD. This may be due to differences in age or gender distribution, socio-demographic or -economic background of subjects between these studies, or by the health care provided for participants in this study. Male gender, HIV-infection, chronic lung disease and shorter duration of ART were independent factors associated with anthropometric deficiency. Anthropometric abnormality, regardless of type or severity, was most common among subjects with both HIV-infection and chronic lung disease, which could be caused by a synergistic effect on anthropometric status. ART is not a sufficient treatment of anthropometric deficiencies. Thus, affected children and adolescents are in need of extra nutritional support. It is essential that nutritional status is assessed and that deficiencies or complications are treated with close follow-up, to avoid progression of anthropometric deficiency, which in turn is a predictor of HIV-progression, morbidity and mortality. Any health care provider for these individuals should be aware of potential anthropometrical deficiencies and their potential consequences. The ultimate solution would be prevention of primary infection in women and vertical transmission of HIV-infection.

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8 Appendix

Table 1. *Demographic and clinical parameters of the study participants.*

Characteristic	HIV with CLD	HIV without CLD	HIV uninfected	HIV without CLD versus HIV negative, P-value	HIV positive with CLD versus without CLD, P-value
Total, n	213	51	97		
Demographics					
Male, n (%)	117 (54.9)	18 (35.3)	50 (51.5)	0.082	0.013
Female, n (%)	96 (45.1)	33 (64.7)	47 (48.5)	0.082	0.013
Age, median (IQR)	15 (12-17)	15 (12-18)	10 (7-12)	0.000	0.573
Age groups, n (%)				0.000	0.380
6-9 years	24 (11.3)	8 (15.7)	48 (49.5)		
10-12 years	51 (23.9)	10 (19.6)	38 (39.2)		
13-16 years	78 (36.6)	14 (27.5)	11 (11.3)		
17-19 years	60 (28.2)	19 (37.3)	0		
Anthropometric data					
Height-for-age z-score, median (IQR)	-1.88 (-2.56- -1.13)	-1.29 (-1.75- -0.76)	-0.59 (-1.12- -0.06)	0.000	0.000
Stunted (height-for-age z-score <-2 SD), n (%)	97 (45.8)	8 (15.7)	4 (4.1)	0.024	0.000
Severely stunted (height-for-age z-score <-3SD), n (%)	30 (14.2)	1 (2.0)	0		0.014
Weight-for-age z-score, mean (SD)	-1.73 (± 0.95)	-1.00 (0.57)	-0.29 (± 0.98)	0.054	0.052
Underweight (weight-for-age z-score <2SD), n (%)	9 (37.5)	0	2 (4.2)	1.000	0.070
Severely underweight (weight-for-age z-score <-3SD), n (%)	3 (12.5)	0	1 (2.1)		0.555
BMI-for-age z-score, median (IQR)	-1.21 (-1.89- -0.45)	-0.34 (-0.87- -0.48)	-0.19 (-0.66-0.43)	0.656	0.000
Low BMI (BAZ<-2 SD), n (%)	44 (20.8)	1 (2.0)	1 (1)	1.000	0.001
Severely low BMI (BAZ<-3SD), n (%)	8 (3.8)	0	1 (1)		0.222
Clinical data					
FEV1 z-score, median (IQR)	-1.86 (-2.43- -1.43)	0.54 (0.25-0.79)	-0.24 (-0.61- -0.32)	0.000	0.000
Past history of tuberculosis, n (%)	72 (33.8)	8 (15.7)			0.017
Anemia, n (%)	46 (30.3)	14 (27.5)	9 (9.8)	0.009	0.728
Age when ART was initiated, median (IQR)	8 (5-11)	8 (6-11)			0.573
Years treated with ART, median (IQR)	6.4 (3.9-8.2)	6.9 (4.1-8.7)			0.365
HIV viral load (copies/ml), median (IQR)	372 (46-8970)	278 (39-2360)			0.150
HIV viral load >1000 copies/mL, n (%)	88 (41.3)	15 (29.4)			0.150
CD4 count (cells/mm^3), median (IQR)	565 (343-755)	680 (439-917)			0.026
CD4 t-cell levels (count)					0.372
None to mild immunodeficiency (≥350), n (%)	157 (73.7)	41 (80.4)			
Advanced immunodeficiency (<350), n (%)	56 (26.3)	10 (19.6)			

%: of valid total within characteristic groups, etc. HIV positive with CLD

Table 2 *Characteristics of subjects with anthropometric abnormalities*

	HIV with CLD n (%)*			HIV without CLD n (%)*			HIV uninfected n (%)*		
Characteristic	**Stunted**	**Underweight**	**Low BMI**	**Stunted**	**Underweight**	**Low BMI**	**Stunted**	**Underweight**	**Low BMI**
Total	97	9	44	8	0	1	4	2	1
Male	66 (68.0)	6 (66.7)	34 (77.3)	5 (62.5)		1	1 (25.0)	2	1
Female	31 (32.0)	3 (33.3)	10 (22.7)	3 (37.5)			3 (75.0)		
Age groups									
6-9 years	13 (13.4)	9 (100)	2 (4.5)	1 (12.5)			4 (100)	2 (100)	1
10-12 years	20 (20.6)		9 (20.5)	1 (12.5)			0		
13-16 years	41 (42.3)		20 (45.5)	2 (25.0)			0		
17-19 years	23 (23.7)		13 (29.5)	4 (50.0)		1	0		
Anemia	21 (30.0)	0	8 (26.7)	4 (50.0)		1	1 (33.3)	0	0
HIV viral load									
>1000 copies/mL	46 (47.4)	4 (44.4)	18 (40.9)	3 (37.5)		1			
<1000 copies/mL	51 (52.6)	5 (55.6)	26 (59.1)	5 (62.5)					
CD4 t-cell levels (count)									
None to mild immunodeficiency (≥350)	68 (70.1)	5 (55.6)	36 (81.8)	5 (62.5)					
Advanced immunodeficiency (<350)	29 (29.9)	4 (44.4)	8 (18.2)	3 (37.5)		1			

*Number and proportion of subjects within the groups of each anthropometric abnormality with certain characteristic

Table 3 *Analysis of factors associated with being stunted among HIV-infected individuals.*

Characteristic	Level	N (%)*	Crude odds ratio (95% CI)	Adjusted odds ratio (aOR) **	P-value
Sex	Female	34 (27%)	1.0 (ref.)	1.0 (ref.)	
	Male	71 (53%)	3.07 (1.83-5.147)	3.18	0.000
CD4 levels	None to mild immunodeficiency (≥350)	73 (37%)	1.0 (ref.)	1.0 (ref.)	
(CD4 count per mm^3)	Advanced immunodeficiency (<349)	32 (49%)	1.59 (0.911-2.81)	1.58	0.144
HIV VL copies/mL	<1000	56 (35%)	1.0 (ref.)	1.0 (ref.)	
	>1000	49 (48%)	1.73 (1.05-2.88)	1.57.	0.106
Anemia	No anemia	53 (37%)	1.0 (ref.)	1.0 (ref.)	
	Anemia	25 (42%)	1.25 (0.67-2.32)	1.44	0.302
CLD status	No CLD	8 (16%)	1.0 (ref.)	1.0 (ref.)	
	CLD	97 (46%)	4.53 (2.03-10.10)	3.97	0.001

* Number and proportion of participants who were stunted in each group.
**As default adjusted for age and gender. HIV viral load and CD4 levels were additionally adjusted for CLD status. All other covariates were not adjusted for because of collinearity.

Table 4 *Analysis of the association between HIV infection and being stunted.*

Characteristic	Level	N (%)	Crude odds ratio (95% CI)	Adjusted odds ratio (aOR)**	P-value
HIV infection	No HIV infection	4 (4.1 %)	1.0 (ref.)		
	HIV infection*	8 (15.7 %)	4.326 (1.24-15.15)	6.34	0.003

* HIV infection without CLD
** Adjusted for age, sex and CLD

Table 5 Analysis of factor associated with low BMI (BMI-for-age z-score <-2 SD) in HIV-infected individuals.

Characteristic	**Level**	**N (%)***	**Crude odds ratio (95% CI)**	**Adjusted odds ratio ****	**P-value**
Sex	Female Male	10 (8%) 35 (26%)	1.0 (ref.) 4.21 (1.98-8.92)	1.0 (ref.) 3.96.	 0.002
CD4 levels (CD4 count per mm^3)	None to mild immunodeficiency (≥350) Advanced immunodeficiency (<349)	36 (18.2%) 9 (13.8%)	1.0 (ref.) 0.72 (0.33-1.59)	1.0 (ref.) 0.58	 0.195
HIV VL copies/mL	<1000 >1000	26 (16%) 19 (19%)	1.0 (ref.) 1.19 (0.62-2.28)	1.0 (ref.) 0.87	 0.695
Anemia	No anemia Anemia	22 (15%) 9 (15%)	1.0 (ref.) 0.99 (0.43-2.29)	1.0 (ref.) 0.86	 0.753
CLD status	No CLD CLD	1 (2%) 44 (21%)	1.0 (ref.) 13.1 (1.76-97.45)	1.0 (ref.) 11.47	 0.019

* Number and proportion of participants who had low BMI in each group.
**As default adjusted for age and gender. CLD status was additionally adjusted for anemia. HIV viral load and CD4 levels were adjusted for CLD status. All other covariates were not adjusted for because of collinearity.

9 Overview of GRADE evaluation of five important references

<table>
<tr><td colspan="3">Referanse: Rylance S, Rylance J, McHugh G, Majonga E, Bandason T, Mujuru H, et al. Effect of antiretroviral therapy on longitudinal lung function trends in older children and adolescents with HIV-infection. PLoS One. 2019;14(3):e0213556-e.</td><td>Study design: Cohort study
Grade – quality **</td></tr>
<tr><td>Outcome</td><td>Material and methods</td><td>Resultater</td><td>Discussion/comments/checklist</td></tr>
<tr><td>1) Assess evolution of lung function in HIV-infected children
2) Assess effect of ART on lung function

Conclusion
1) Improvement of lung function within, but not beyond, 2 years after ART initiation.

2) Positive contributors of lung function is early ART initiation and</td><td>Population:
Prospective 2 year follow-up of 6-16 year old HIV-infected subjects recruited from several clinics in Harare, Zimbabwe. HIV-established subjects (n=197) who had been treated for >6 months were recruited from a public clinic, while ART-naïve subjects (n=271) were recruited from seven public clinics in Harare.

Cohorts:
Data was compared between ART-naïve cohort and ART-established cohort.

Main outcomes:
Z-scores of forced expiratory volume (FEV1z) and forced vital capacity</td><td>Main finding
In the ART- naïve cohort, FVCz was estimated to increase by 0.09 per year the first two years after ART initiation. This improvement was not discovered in the ART-established cohort.
Duration of ART after initiation was associated with change in FVC z-score, but did not contribute with a significant improvement of FEV1 z-scores.
Age at ART initiation was negatively associated with FEV1 and FVC for both cohorts. FVC z-score decreases by 0.04 for every year ART initiation is delayed.

Between exposed/unexposed:
See main finding.</td><td>Checklist:
• Is the outcome clearly defined?
Yes
• Are the groups collected from the same population? (selection bias)
Yes, both cohorts originates from clinics in Harare. The inclusion criteria are the same, with exception of different time of ART initiation.
• Were the groups comparable in regard of important background factors? (selection bias)
Yes, they were selected based on age, regional affiliation, diagnosis of HIV infection and absence of acute illness.
• Were the exposed subjects representative for a defined population?</td></tr>
</table>

improved nutritional status. **Country** Zimbabwe **Year of data collection** 2013 - 2015	(FVCz), time on ART and age at initiation of ART. **Important confounding factors:** Age, sex **Statistical methods:** Linear mixed-effects regression model* of longitudinal data was performed to assess the relationship between lung function (FEV1 and FVC z-scores) and explanatory co-variates like time on ART, age at initiation of ART and BMI z-scores. Co-variates were evaluated by likelihood ratio comparison to select parameters contributing to significantly improved model fit.	**Rate/proportion//ratio/rate difference:** Not estimated. **How strong is the association (RR)?** Not estimated using RR, but reported with coefficients of change in z-scores. **What is the absolute risk reduction (ARR)?** Not estimated. **CI (wide/narrow):** 95% confidence intervals were only calculated with coefficients for change in FVC and FEV z-scores. 95% CI for effect of time on ART on FVC is 0.01 to 0.18. 95% CI for effect of age at ART initiation on FVC z-score was -0.09 to 0.00 for both cohorts. **Dosage-response?** Not relevant **Secondary findings** Body mass index was positively associated with FEV1 and FVC z-scores in both cohorts.	They are representative for HIV infected children and adolescents receiving ART in that region. • **Was exposition and outcome measured similarily and reliably (validated) in these two groups? (Classification bias)** Yes • **Is the measurer of results (endpoints) blinded for group affiliation?** Not relevant. • **Was the study prospective?** Yes, the patients were followed 2 years from enrolment. • **Were enough subjects in the cohort followed up? (Attrition bias/follow-up-bias)** Yes • **Was dropout analyses performed? (Eval. attrition bias)** Yes. Out of 385 recruited subjects not on ART, 78 did not start ART during study period. Of the remaining 307, 271 had at least one valid spirometry assessment. Of 202 recruited subjects on ART, 5 did not have at least one valid spirometry assessment. • **Was follow-up time long enough to detect positive/negative outcomes?**

			Yes. The follow-up time was long enough to estimate per year changes in lung function depending on explanatory variables like duration of ART and nutritional status. • **Has confounding factors been taken into account regarding design/implementation/analyses?** Yes. Z-scores of lung function were specific for race and sex, and adjusted for height and age. • **Do you trust the results?** **-Bradford Hills criteria (time sequence, dose-response gradient, biological plausibility, consistency….)** Yes. • **Can the results be transferred to the general population?** No, they are depending on the specific setting and inclusion criteria of this study. • **Other literature that confirms/weakens the results?** Similar studies support a positive correlation between lung function and nutrition. Findings of decreased lung function per year after delay of ART initiation was supported by WHO guidelines stating ART

			should be given to all children, adolescents and adults after diagnosis of HIV infection. • **What does the findings mean for change of practice?** It strengthens the importance of anthropometric measurements before and during ART, as a simple method of monitoring treatment effect of ART when more advanced methods are unavailable in resource-limited settings. **What does the authors discuss as:** • **Strengths** Spirometry was conducted by experienced staff according to guidelines, with 88 % in each cohort meeting these standards. • **Weaknesses** 1) Analysis combined two cohort studies with different follow-up schedules. Clinical data were different for each cohort. Analysis is therefore restricted to lung function and anthropometry. 2) Because of restricted measurements across cohorts, they were only compared at one timepoint after enrolment.

			3) Measurement error and physiological day-to-day fluctuation affects individual spirometry variations.

Referanse: Weigel R, Phiri S Fau - Chiputula F, Chiputula F Fau - Gumulira J, Gumulira J Fau - Brinkhof M, Brinkhof M Fau - Gsponer T, Gsponer T Fau - Tweya H, et al. Growth response to antiretroviral treatment in HIV-infected children: a cohort study from Lilongwe, Malawi. 2010(1365-3156 (Electronic)).			**Study design: Cohort study** Grade – quality: **
Outcome	Material and methods	**Resultater**	**Discussion/comments/checklist**
Examine and assess response of anthropometric status to ART in children treated at a public-sector clinic in Malawi >12 months. **Conclusion** Normal growth were not obtained despite sustained growth response to ART. Earlier diagnosis and treatment could improve growth response. **Country** Malawi **Year of data collection**	**Population:** All (n=497) children younger than 15 years who initiated ART at Kamuzu Central Hospital between January 2001-December 2006 were included and followed up until March 2008. **Cohorts:** Males, females, age (<2, 2-4, 5-9, ≥10 years), baseline WAZ and HAZ (≤−3, >−3 to ≤−2, >−2 to ≤−1, >−1), time of initiation of ART (2001 to June 2004, June 2004 to Dec 2004, Jan 2005 to Dec 2005, 2006) **Main outcomes:**	**Main finding** Baseline WAZ and HAZ were the most important determinants of growth trajectories on ART, of which low z-score at ART initiation was the most important contributor for growth response. **Between exposed/unexposed:** Not relevant **Rate/proportion//ratio/rate difference:** Not estimated. **How strong is the association (RR)?** Not estimated using RR, but reported with coefficients of change in z-scores from ART initiation to 2	**Checklist:** • **Is the outcome clearly defined?** Yes. • **Are the groups collected from the same population? (selection bias)** Yes. The samples were collected from children and adolescents attending the public clinic in Lilongwe. • **Were the groups comparable in regard of important background factors? (selection bias)** Yes, they were selected based on age, diagnosis of HIV infection and receiving of ART. • **Were the exposed subjects representative for a defined population?**

2001-2008	Prevalence of underweight and stunting presented as age- and sex-adjusted z-scores <-2. Response of anthropometric status to ART in children after 2 years of treatment. **Important confounding factors:** Age, sex, baseline anthropometric z-score, HIV clinical stage, degree of immunodeficiency, time of data collection, **Statistical methods:** Medians and interquartile ranges of weight-for-age z-score (WAZ), height-for-age z-score (HAZ) and CD4 percentage calculated from ART initiation up to 2 years. The trajectories of anthropometric measurements were analyzed using fractional polynomial regression with WAZ and HAZ as outcomes in mixed-effect models. Co-variables were age, sex, baseline WAZ and HAZ, baseline WHO clinical stage of HIV, degree of immunodeficiency and time period of ART initiation. Multinomial regression models were used with stage of HIV disease and degree of immunodeficiency as outcome, and age, sex, year of ART	years after. Low baseline z-scores had the strongest association, see CI below. **What is the absolute risk reduction (ARR)?** Not estimated **CI (wide/narrow):** 95% confidence intervals were calculated for coefficients for main effects on WAZ and HAZ depending on baseline characteristics. 95% CI for the lowest z-scores were -3.14 to -2.79 for WAZ and -3.41 to -2.94 for HAZ. **Dosage-response?** Not relevant **Secondary findings** Neither underweight nor stunted children achieved normal growth after 2 years on ART.	Yes, for HIV infected children and adolescents receiving ART in that region. • **Was exposition and outcome measured similarly and reliably (validated) in these two groups? (Classification bias)** Yes. • **Is the measurer of results (endpoints) blinded for group affiliation?** Not relevant. • **Was the study prospective?** Yes, the patients were followed from ART initiation to at least 24 months after. • **Were enough subjects in the cohort followed up? (Attrition bias/follow-up-bias)** There was a high dropout rate. Nevertheless, the sample size was adequate. • **Was dropout analyses performed? (Eval. attrition bias)** Yes. 21 (4.3%) children died, 140 (28.2%) were lost to follow-up and 136 (27.4%) were transferred to another treatment facility. • **Was follow-up time long enough to detect positive/negative outcomes?**

	initiation and WAZ at initiation of ART as predictors. Missing data were imputed by randomly sampling from the corresponding predictive multinomial distribution.		Yes. The follow-up time was long enough to detect trends in z-score trajectories beyond 12 months, according with the predefined outcome. It was not long enough to define how long it takes to reach normal z-scores. • **Has confounding factors been taken into account regarding design/implementation/analyses?** Yes. They were adjusted for in statistical analysis. • **Do you trust the results?** **-Bradford Hills criteria (time sequence, dose-response gradient, biological plausibility, consistency….)** Yes. • **Can the results be transferred to the general population?** No, they are depending on the specific setting and inclusion criteria of this study. • **Other literature that confirms/weakens the results?** Similar studies from the African region shows similar findings, although American and European studies shows more promising catch-up growth. • **What does the findings mean for change of practice?**

			It strengthens the importance of anthropometric measurements before and during ART, as a simple method of monitoring treatment effect of ART when more advanced methods are unavailable in resource-limited settings. **What does the authors discuss as:** • **Strengths** The study is responding to a need for further assessment of growth response of ART longer than 12 months, as there were few studies from before initiation of the study. • **Weaknesses** 1) High proportion of missing data 2) High proportion were lost to follow-up or transferred to another clinic 3) Median age was 8 years at ART initiation, and results are therefore less applicable to infants and young children

Reference: Hillesheim E, Lima Lr Fau - Silva RCR, Silva Rc Fau - Trindade EBSM, Trindade EB. Dietary intake and nutritional status of HIV-1-infected children and adolescents in Florianopolis, Brazil. 2014(1758-1052 (Electronic)).			**Design: Cross sectional study** Grade - quality ****
Outcome	**Material and methods**	**Results**	**Discussion/comments/checklist**
1) To evaluate the dietary intake and nutritional status of a group of HIV-infected children and adolescents living in Florianópolis, Brazil 2) Determine their associations with CD4+ T-cell count and viral load. **Conclusion** Despite using highly active antiretroviral therapy (HAART) and a high frequency of adequate nutritional status, the sample showed negative mean z-scores for BMI-for-age and height-for-age and high energy intake. Subjects had low intake of important nutrients for immune funtion	**Population:** 49 HIV-1-infected children and adolescents between 7 and 17 years of age who received highly active antiretroviral therapy (HAART) at Hospital Infantil Joana de Gusmão. Inclusion criteria were having acquired HIV by mother-to-child transmission, having both clinical and laboratory data in medical records, being 7-17 years old, having no signs or symptoms of chronic diseases and no use of medications that altered body compositon or nutritional status. **Main outcome variables:**	2.0 % were underweight, 6.1% were stunted. Viral load was undetectable in 55.1% of subjects, and only two showed value above 10 000 copies/ml of HIV RNA. 61.5% of subjects were in early stage of the disease, 38.5% were moderately og severely symptomatic. Total energy intake (TEI) was above estimated energy requirements (EER) in 89.8% of subjects. The mean macronutrient intake was adequate for carbohydrate and protein, and below recommendation for total fat. Protein intake was on average 408% above the recommendation of estimated average requirements (EAR). Frequency of inadequate intake depended on the nutrient: 100% for polyunsaturated fat intake, 57.1% for cholesterol, 40.8% for fibre, 61.2% for calcium,	**Checklist*** • **Is the outcome clearly described?** Yes. Outcome variables were micro- and macronutrient intake, nutritional status and clinical measurements of HIV infection (CD4+ T-cell count and viral load). • ***Was the population the sample was selected from clearly defined?** Yes, they were selected among patients with HIV-1-infection from a hospital in Brazil. • ***Was the sample representative for the population group?** Yes, for HIV-1-infected children and adolescents receiving HAART in the same are. The study reports that similar studies from Brazil shows much higher prevalence of stunting, but similar prevalence of underweight. • **Was the data sampling standardized?** Yes. Data on nutritional status was reported by z-scores. • ***Was the response rate high enough?** The sample size was In total 19 out of 68 eligible subjects didn´t participate due to lack of interest/time or by not attending follow-

(polyunsaturated fat, vitamin C) and for growth (calcium). Lower energy intake was related to viral suppression and immune preservation. Results suggest it is necessary to assess dietary intake of HIV-infected children and adolescents frequently to allow for appropriate growth and immune response. **Country** Brazil **Year of data collection**	Diatary intake, nutritional status, CD4+ T-cell count, HIV viral load **Important confounding variables:** Gender and age **Statistical methods:** Normality assessed by Shapiro-Wilk test and graph representation. Non-parametric data were normalized using log10. Descriptives were used for data reporting. Inferential statistics included t-Student test for independent samples, chi-Square test, U-Mann-Whitney test, Spearman correlation and multiple linear regression analysis. Multiple analyses were adjusted for age and gender. The variables were introduced one at a time into the models by the *enter* procedure. Significance level set at 0.05.	34.7% for vitamin A and 26.5% for vitamin C. All subjects had adequate iron intake. Moderate to weak correlations among clinical measurements of HIV infection, dietary intake and nutritional status were found ($p < 0.05$). After adjustment for energy intake, all these correlations were not significant. In multiple regression analysis, energy intake was positively correlated with viral load and inversely correlated with CD4+ T-cell count when adjusting for gender and age.	ups. No subjects withdrew from the evaluation during the study. • **Are objective criteria used for assessment of outcomes? (Classification bias)** Yes, including measures of CD4+ T-cell count, HIV viral load, z-scores of anthropometric indicators. • **Were adequate methods used in data analysis?** Yes. • **Were the inclusion criteria clearly defined?** Yes. • **Are there any prognostic / confounding factors described / taken into account in design/analysis?** Yes. Gender and age are adjusted for in data analyses. Inclusion criteria included no other chronic disease or medications causing nutritional deficit. • **Other literature support the results?** The study compares its results with several other similar studies. Some of them support the findings, others not. **What does the authors discuss as** • **Strengths** No dropouts. • **Weknesses** The cross-sectional design does not allow for the establishment of a cause and effect relationship between the variables. Small sample size requires caution in interpreting

2010			the results, and also prohibits analyses using age subgroups, given the age range of suibjects. Dietary intake is influenced by regional and social contexts, and may only represent the local conditions. The study did not investigate the relationship between socioeconomic factors and dietary intake or nutritional status.

* Source for GRADE checkpoints: https://www.fhi.no/globalassets/dokumenterfiler/skjema/brukererfaring/k-handbok_11_vedlegg2_sjekklister.pdf

Reference: Muenchhoff M, Healy M, Singh R, Roider J, Groll A, Kindra C, et al. Malnutrition in HIV-Infected Children Is an Indicator of Severe Disease with an Impaired Response to Antiretroviral Therapy. AIDS Res Hum Retroviruses. 2018;34(1):46-55.			**Design: Cohort study** Grade - quality ****
Outcome	**Material and methods**	**Results**	**Discussion/comments/checklist**
Describe and compare levels of microbial translocation and immune activation and exhaustion in HIV-uninfected and HIV-infected ART-naïve children presenting with or without severe acute malnutrition (SAM) and after 48 weeks of ART.	**Population:** Children aged 2 months to 12 years in four groups: HIV infected with (n=32) and without SAM (n=41), HIV-uninfected controls with (n=15) and without SAM (n=19). The HIV infected groups were prospectively followed up after 48 weeks after initiation of ART.	**Main finding** Microbial translocation, T cell activation and exhaustion were significantly increased in HIV-uninfected children with SAM compared to HIV-uninfected children without SAM. Immune activation was significantly elevated in HIV-uninfected children with SAM compared to healthy controls.	**Checklist:** • **Is the outcome clearly defined?** Yes • **Are the groups collected from the same population? (selection bias)** Yes. The samples were collected from children and adolescents attending the public clinic in Lilongwe.

And further use this in guidance of treatment interventions towards this critically ill patient group. Hypotheses: 1) Malnutrition is associated with microbial translocation, immune activation and immune exhaustion in HIV-infected and uninfected children 2) Malnutrition, microbial translocation and immune activation are associated with detrimental treatment outcome in HIV-infected children on ART	Patients were recruited from the King Edward VIII Hospital, Durban, South Africa and from the Ithembalabantu Clinic, Umlazi, Durban, South Africa in different time periods between July 2012 and March 2015. **Main outcome variables:** Markers of microbial translocation (bacterial 16sDNA), intestinal damage (iFABP), monocyte activation (sCD14), T-cell activation (CD38, HLA-DR) and immune exhaustion (PD1). **Important confounding variables:** Age, sex, tuberculosis status **Statistical methods:**	In HIV-infected children microbial translocation, immune activation, and exhaustion was strongly increased but did not differ by SAM-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. **Between exposed/unexposed:** Not relevant **Rate/proportion//ratio/rate difference:** Not estimated. **How strong is the association (RR)?** Not relevant **What is the absolute risk reduction (ARR)?** Not relevant **CI (wide/narrow):** Not estimated. **Dosage-response?** Not relevant	• **Were the groups comparable in regard of important background factors? (selection bias)** Yes, they were selected based on age, diagnosis of HIV infection and receiving of ART. • **Were the exposed subjects representative for a defined population?** Yes, to individuals infected with HIV with or without SAM in the specified age range. • **Was exposition and outcome measured similarily and reliably (validated) in these two groups? (Classification bias)** Yes. • **Is the measurer of results (endpoints) blinded for group affiliation?** Not relevant. • **Was the study prospective?** Yes, the patients were followed for 48 weeks after initiation of ART. • **Were enough subjects in the cohort followed up? (Attrition bias/follow-up-bias)** Yes. Subjects were overenrolled to adjust for potential loss to follow-up. • **Was dropout analyses performed? (Eval. attrition bias)**
Conclusion			

Severe acute malnutrition is an indicator of severe disease associated with worse prognosis and impaired immune recovery in HIV-infected children on ART. SAM is also associated with increased microbial translocation, immune activation and immune exhaustion in HIV-uninfected children. Immune activation and microbial translocation are associated with impaired immune recovery in HIV-infected children on ART.	Anthropometric z-scores were calculated using WHO Anthro software. Baseline characteristics were compared between study groups using Kruskal-Wallis ANOVA with Dunn´s correction for multiple comparisons. Spearman correlations were used to explore bivariate associations. Kaplan-Meier survival curves were compared by the log-rank test. Chi-Square test was used to assess difference in prevalence of tuberculosis and viral suppression between study groups. Multiple regression analysis was performed using a Gaussian generalized linear model (GLM) to assess associations between clinical and immunological covariates with CD4+ T-cell immune activation.	**Other findings** SAM was associated with increased mortality rates early after ART initiation.	Yes, although it mentions only HIV infected subjects: 5 were deceased and 1 lost to follow-up among HIV+SAM+. 1 was deceased and 4 lost to follow up among HIV+SAM-. • **Was follow-up time long enough to detect positive/negative outcomes?** Yes. The follow-up time was long enough to detect changes in biological and anthropometric status. • **Has confounding factors been taken into account regarding design/implementation/analyses?** Yes. • **Do you trust the results?** **-Bradford Hills criteria (time sequence, dose-response gradient, biological plausibility, consistency….)** Yes. • **Can the results be transferred to the general population?** No, they are depending on the specific setting and disease status of the study subjects. • **Other literature that confirms/weakens the results?** The authors tells us there is limited knowledge of the effect of SAM microbial translocation, immune activation and –exhaustion in HIV-infected children. The study refers to studies showing similar findings regarding for example the associations between malnutrition and immune activation. It also refers to studies

Country South Africa **Year of data collection** 2012-2015	The use of least absolute shrinkage and selection operator (LASSO) for variable selection was selected due to small sample size in relation to number of covariates, but also to handle a certain degree of multicollinearity. All variables were scaled. Missing values were excluded from analysis.		confirming the same finding that immune activation is a stronger predictor for immune recovery than malnutrition. • **What does the findings mean for change of practice?** Authors hope the study may help to inform about the pathogenesis of malnutrition and HIV infection and guide future treatment interventions. **What does the authors discuss as** • **Strengths** Not mentioned • **Weknesses** 1) There are large differences between study groups, especially regarding younger age and increased comorbidities in subjects with SAM. The study explains this by malnutrition being an important indicator of illness with substantial immunological alterations. 2) Low number of participants with complete available data. The results should be validated in larger cohorts. 3) This study is observational and can therefore only describe associations.

Reference: Jesson J, Masson D, Adonon A, Tran C, Habarugira C, Zio R, et al. Prevalence of malnutrition among HIV-infected children in Central and West-African HIV-care programmes supported by the Growing Up Programme in 2011: a cross-sectional study. BMC Infect Dis. 2015;15:216.			**Design: Cross sectional study** Grade - quality ** **Discussion/comments/checklist**
Outcome Assess prevalence and associated factors of acute and chronic malnutrition among HIV-infected children followed up in the HIV-care programmes in Central and West Africa funded by the Growing Up programme. **Conclusion** There is high prevalence of malnutrition for HIV-infected children in sub-Saharan Africa, even in an HIV care programme supposed to have high standards for care. Active management of HIV-infected children with routine anthropometric measurements to allow earlier detection of malnutrition, also as a measurement of treatment response, and	**Material and methods** **Population:** 1350 HIV-infected children aged 2-19 years were enrolled in HIV-care programmes in the time period from Sept to Dec 2011 from 12 health centers in Benin, Burundi, Cameroon, Côte d'Ivoire, Mali, Chad and Togo. Inclusion criteria: HIV infection regardless of present ART, with available data for gender, age, weight and height, and with at least one attendance to the programme during the study period. **Main outcome variables:** 1) Acute malnutrition defined by WHZ or BAZ < -2 SD and HAZ ≥ -2 SD; (2) chronic	**Results** Main finding: The prevalence of acute, chronic and mixed malnutrition was 9%, 26% and 7%, respectively. More than half did not receive any nutritional support during the study or within 6 months prior. Boys dispose of significantly increased prevalence than girls regarding all types of malnutrition. Prevalence of chronic or mixed malnutrition was more prevalent in younger (2-5 years) than older children (5-10 years). Children with severe immunodeficiency or recently initiation of ART had higher risk for acute or mixed malnutrition. **Between exposed/unexposed:** Not relevant **Rate/proportion//ratio/rate difference:** Crude and adjusted odds ratios were estimated for potential	**Checklist:** • **Is the outcome clearly described?** Yes. • ***Was the population the sample was selected from clearly defined?** Yes • ***Was the sample representative for the population group?** Yes and no. The study claims they included nearly all children enrolled in the 12 centres participating in the Growing Up programme, making them as best as possible representative of a similar population in HIV care programmes in West and Central Africa. Nevertheless, they also states most data are collected from urban sites, making them less representative for rural regions. Due to exclusion of children aged <2 years, the sample is not representative of a birth cohort of HIV-infected children in sub-Saharan Africa, leading to an underestimation of the prevalence of malnutrition. • **Was the data sampling standardized?** Yes. Data on nutritional status was reported by z-scores. • ***Was the response rate high enough?**

could prevent morbidity and mortality risks. **Country** Benin Burundi Cameroon Côte d'Ivoire Mali Chad Togo **Year of data collection** 2011	malnutrition defined by WHZ/BAZ ≥ -2 SD and HAZ < -2 SD, and (3) mixed malnutrition as WHZ/BAZ < -2 SD and HAZ < -2 SD **Important confounding variables:** Age group, sex, country, immunodeficiency, malnutrition history, duration on ART, orphan status and cotrimoxazole prophylaxis. **Statistical methods:** Subject characteristics were presented by age group, type of malnutrition. Prevalence of malnutrition was calculated using the three anthropometric indicators (HAZ, BAZ and WHZ) with 95 % CI. Comparisons for qualitative and quantitative variables between groups were made using Pearson coefficient test and	explanatory factors of malnutrition. **How strong is the association (RR)?** Only odds ratio of explanatory factors on malnutrition was calculated in the statistical analyses. Example of chronic malnutrition: boys vs. girls (aOR = 1.56, 95% CI = [1.20–2.03], older (5-10 years) vs. younger (2-5 years) children (aOR = 0.61, 95% CI = [0.38–0.99]). **What is the absolute risk reduction (ARR)?** Not estimated. **CI (wide/narrow):** 95% confidence intervals were calculated for odds ratios of explanatory factors. See above. **Dosage-response?** Not relevant **Other findings:** Orphan status was not associated with malnutrition.	Yes. The sample size was large even after exclusion of missing data. The study was cross-sectional, excluding the opportunity of dropouts. • **Are objective criteria used for assessment of outcomes? (Classification bias)** Yes, including anthropometric and immunological parameters. • **Were adequate methods used in data analysis?** Yes. • **Were the inclusion criteria clearly defined?** Yes. • **Are there any prognostic / confounding factors described / taken into account in design/analysis?** Yes. Regression analysis adjusts for the beforementioned confounding variables. • **Other literature support the results?** The authors claim it difficult to compare findings with other studies due to difference in study population and definitions of malnutrition. Nevertheless, the authors do refer to similar studies with higher, similar or lower prevalence of malnutrition depending on subtype. Similar findings of association between gender and malnutrition have been found in other studies. **What does the authors discuss as** • **Strengths**

	Kruskal-Wallis test, respectively. Multinomial regression analysis was performed to assess associations of malnutrition. Missing data were not excluded.		1) The study included nearly all children enrolled in the programme. 2) High quality of data collection with >97% of anthropometric data available. 3) Very few other studies have reported the nutritional practices in HIV-infected malnourished children. It highlights the need to focus on interventions in such children. • **Weknesses** 1) Children had access to pediatric HIV health care, mostly in urban areas where standards of care may be higher of that in rural areas, making the findings hardly relatable for rural settings. 2) Children <2 years were excluded due to lack of respect to inclusion criteria. This age group is known to be especially vulnerable to malnutrition. 50 % of HIV-infected children die within the age of two, excluding the sickest children from the study (survivor bias). Hence, there is an underestimation of the prevalence of malnutrition. 3) There are possible measurement errors in weight and height, although all centers were standard measurement protocol according to WHO recommendations. 4) 20 children with a low weight-for-age were not defined as malnourished and misclassified in the analyses due to the definition of malnutrition used.

			5) The study design does not allow for estimated of causality between malnutrition and explanatory variables.