2016

Risk Factors for Measles among HIV-infected Children in Uganda

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Abstract

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MPH, University of South Florida, 1998

MB CHB, Makerere University, 1992

Dissertation Submitted in Partial Fulfillment

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Doctor of Philosophy

Public Health - Epidemiology

Walden University

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Abstract

Measles remains a major global public health problem. Attainment of high population immunity to measles through vaccination is necessary to control this disease. Children infected with HIV infection often experience secondary measles vaccine failure by 2 years of age, making them susceptible to measles. It is not clear whether HIV-infected children on Highly Active Antiretroviral Treatment (HAART), older than 2 years, have a higher risk of measles than HIV-uninfected children. This retrospective cohort study, guided by the proximate determinants framework, was conducted to compare the risk of measles between HIV-infected children on HAART (exposed) and HIV-uninfected peers (unexposed). The age group with the highest measles susceptibility in the exposed children, which could inform timing for revaccination, was investigated. The role of age at initiation of HAART, low CD4+ count, and undernutrition as predictors of the risk of measles in the exposed children was examined. Univariate, bivariate, and binomial logistic regression analytical procedures were used in data analysis. Results showed no significant difference in the risk of measles between exposed and unexposed children. The age groups 5 to 9 years and 2 to 4 years were the first and second most affected by measles among the exposed children. Undernutrition (stunting) was a significant predictor of measles in exposed children (odds ratio of 4.14, $p = 0.02$), while age at initiation of HAART and CD4+ count prior to measles exposure were not. The study findings provide evidence to inform vaccination policy and nutrition care for HIV-infected children on HAART in Uganda, so as to reduce their risk of measles illness and mortality, thus contributing to positive social change for the children and the country.

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Dedication

This dissertation is dedicated to my late parents, Mr. Frederick Kalibwani

Kaggwa and Mrs. Gertrude Kaggwa, who inspired me to pursue excellence in all that I do

and to never give up on my goals even when faced with challenges; and to my daughters

Rebecca, Noella, and Angel to inspire them to pursue their dreams with excellence.

Acknowledgments

First of all, I would like to thank my husband, Mr. Barnabas Tumusingize, and my daughters Rebecca Zeta Kirabo, Noella Tamar Mwebaza, and Angel Siima Tusiime who have supported me throughout my PhD journey and allowed me to share some time I would be spending with them for my school work. You have been there for me and have been very understanding. Your support has not been in vain.

Further acknowledgements go to my Committee Chair, Dr. Maria Rangel, for the guidance she offered me throughout the dissertation process; and to Dr. Aimee Ferraro, my second committee member for the encouragement, advice, and support during the dissertation journey. I also extend my gratitude to Dr. Susan Nyanzi, the University Research Reviewer that reviewed my proposal and dissertation report.

I extend my sincere gratitude to Walden University for the Commitment to Social Change scholarship awarded to me that enabled me to pay part of my tuition, especially for the course work. Special thanks also go to all the faculty that facilitated the different courses and residencies I undertook in the program. I learned a lot from you and from the courses and residencies that has enabled me to contribute to positive social change in my work as a public health practitioner and epidemiologist.

In a special way I would like to thank my friends including Dr. Juliet Nabyonga-Orem who encouraged me to embark on the PhD in Public Health –Epidemiology, supported and prayed for me throughout the PhD journey; and Mrs. Pamela Tumwebaze that prayed with me, followed the progress I was making step by step, and encouraged me not to give up when the going was tough.

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Chapter 1: Introduction to the Study

Introduction

Although there has been significant reduction in the global measles burden,

measles still caused 158,000 deaths in 2011 (Centers for Disease Control and Prevention

[CDC], 2013; World Health Organization [WHO], 2013a, 2013b). These deaths could be

averted if more than 95% of children were vaccinated with measles vaccine globally

(Moss &Strebel, 2011). Factors that might affect vaccine effectiveness include

programmatic factors such as inadequate cold chain, and host factors such as

malnutrition, presence of maternal measles antibodies, and immune compromising

conditions such as infection with HIV (Uzicanin& Zimmerman, 2011). These factors

result in primary or secondary vaccine failure, making vaccinated children susceptible to

measles (Biellik, 2010; Moss &Strebel, 2011; Uzicanin & Zimmerman, 2011). Children

with vaccine failure could maintain measles transmission, resulting in outbreaks even in

communities with high vaccination coverage (Bellini & Rota, 2011; Moss & Strebel,

2011; Uzicanin & Zimmerman, 2011).

Whereas HIV infection was not a major threat for measles control in the past due

to the short survival time of HIV-infected children (Helfand, Moss, Harpaz, Scott, &

Cutts, 2005), increasing access to Highly Active Antiretroviral Treatment (HAART) is

resulting in longer survival. Thus, HIV-infected children may form a pool of susceptible

individuals that could maintain measles transmission and hinder measles control

(deMoraes-Pinto, 2011; Moss & Strebel, 2011). It is not clear whether treatment with

HAART prevents secondary vaccine failure and modifies the risk of measles among the children on HAART or whether children on HAART are at higher risk of measles than uninfected children. The purpose of this study is to assess whether there is a difference between the risk of measles among HIV-infected children on HAART and that in HIV-uninfected children of the same age group, and any factors that could be influencing the risk of measles in HIV-infected children on HAART. In this chapter, I cover the background of the study, the problem statement, the purpose of the study, and the research questions and hypotheses. I also discuss the theoretical basis for and nature of the study, provide operational definitions of key terms used, and highlight assumptions, the study scope and delimitations, limitations, and significance of the study.

Background

Burden of Measles in Uganda

Measles remains a major global public health problem due to different factors affecting uptake and effectiveness of the measles vaccine (WHO, 2009a). Despite the significant progress in measles control achieved in the last decade, about 355,000 measles cases and 158,000 deaths due to the disease were reported globally in 2011 (CDC, 2013; WHO, 2013a, 2013b). Measles is caused by the measles virus, from the paramyxovirus family; it is so highly infectious that it can infect and cause disease in more than 90% of unvaccinated people (Bellini & Rota, 2011). In Uganda, measles was one of the major causes of under-five mortality in the last century. In 2002, it ranked fourth among the causes of under-five mortality (Nanyunja et al., 2003). However, since 2003 there have

been accelerated efforts to control measles and reduce the associated morbidity and mortality.

An accelerated measles control strategy was implemented between 2003 and 2006. It involved a wide age group catch up campaign targeting all children from 6 months to 14 years old, follow up campaigns targeting children less than 5 years every 3 to 4 years, nationwide case based surveillance and investigation of each suspected case, strengthening routine measles vaccination, and Vitamin A supplementation (Mbabazi et al., 2008). Implementation of the strategy resulted in 93% and 95% reduction in measles cases and deaths respectively in 2006 (Mbabazi et al., 2009) compared to the year 2000 when 43,931 cases of measles were reported, incidence was 320 cases per 100,000 inhabitants, and case fatality ratio was about 5% (Mbabazi et al., 2009; Nanyunja et al., 2003). As a result of the measles control interventions mentioned above, measles' rank among causes of child mortality in Uganda changed from 4th to 10th by 2006 (Uganda Bureau of Statistics [UBOS], Macro International Inc., and MEASURE Evaluation, 2008). The circulation of indigenous measles virus strain D10 (Muwonge et al., 2005) was interrupted by the high immunity levels achieved by successful implementation of the accelerated measles control strategy. Sporadic outbreaks between 2006 and 2009 were caused by imported strains of measles virus (Baliraine et al., 2011; Mbabazi et al., 2009). Outbreaks linked to imported strains were an indication of accumulation of children susceptible to measles in the community due to either nonvaccination or vaccine failure. Transmission of measles was quickly reestablished among the susceptible children when a measles virus strain was imported into the community. Between 2006

and 2009, 1,053 measles cases were confirmed by the measles laboratory (Baliraine, et al., 2011). However, from 2010 to 2013 there was an upsurge in measles cases; deaths and outbreaks were experienced in several districts in Uganda involving children less than 5 years of age as well as older children and young adults (Mwesigye, 2012; WHO, 2013a). Outbreaks occurred even in areas reporting very high vaccination coverage (Ministry of Health Uganda, 2013). In January 2013, an outbreak of measles was confirmed in Hoima district only 3 months after a successful nationwide mass measles vaccination campaign targeting children less than 5 years old; about half of the confirmed measles cases were among children less than 5 years old, and over 20% had a history of vaccination with at least one dose of measles vaccine (Ministry of Health Uganda, 2013). A total of 1,313; 3,312; and 720 measles cases were reported in 2010, 2011, and 2012 respectively (National Health Management Information System database; WHO, 2013c).

Measles Vaccination and Vaccine Effectiveness

In Uganda, measles vaccine is administered to children as part of the routine immunization program at 9 months of age. A second opportunity for vaccination is provided to all children less than 5 years old every 3 to 4 years through supplementary mass vaccination campaigns (Mbabazi et al., 2008; Nanyunja et al., 2003). Measles vaccine coverage remains inadequate in Uganda in spite of national efforts to strengthen routine immunization. According to a population-based Demographic and Health Survey conducted in Uganda in 2011, the measles vaccination coverage in Uganda in 2010 was 75.8%, showing a 12.8% increase from the coverage in 2006 (Uganda Bureau of Statistics and ICF International Inc., 2012). Although measles vaccine effectiveness

among children vaccinated at 9 months is expected to be about 85%, it can be affected by different program factors such as inadequate cold chain maintenance and inappropriate method of administration. The measles vaccine effectiveness can also be affected by host factors such as presence of maternal measles antibodies at vaccination, the nutrition status of children at vaccination, malaria infections, and immune compromising conditions such as infection with HIV, among others (Kizito et al., 2013; Moss & Strebel, 2011; Uzicanin & Zimmerman, 2011). Mupere et al. (2006) found measles vaccine effectiveness among children in Kampala, Uganda to be 74%, however they did not ascertain the specific factors that were contributing to this lower than expected effectiveness. Kizito et al. (2013) found that by 1 year of age, only 75% of infants in their study in Uganda had protective level of measles antibodies. Given that not all vaccinated children at 9 months of age achieve an adequate antibody response, coverage of more than 95% is required for herd immunity to measles to be achieved in any community (Moss & Strebel, 2011). High prevalence of one or several of the factors affecting vaccine effectiveness can result in a significant number of vaccinated children remaining susceptible to measles.

Burden of HIV in Uganda

Uganda had an HIV prevalence of 7.3% among all people 15 to 49 years old, and 8.3% among women in the reproductive age group between 15 and 49 years in 2011 (Ministry of Health & ICF International, 2012). This is quite high compared to some countries neighboring Uganda within the East African region, such as Ethiopia, which had a prevalence among people 15 to 49 years of 1.4%, Eritrea with prevalence of 0.6%,

and Rwanda at 2.9% (UNAIDS, 2012). HIV prevalence in the general population equal to or greater than 2% is considered high (Ferrand et al., 2011), and HIV is considered hyperendemic in countries where the prevalence in the general population exceeds 15% (UNAIDS/WHO/SACEMA Expert Group on Modeling the Impact and Cost of Male Circumcision for HIV Prevention, 2009). It was estimated that 96,700 pregnant women were HIV-infected, and that about 20,600 children were born with HIV infection in Uganda in 2011, mainly due to inadequate coverage of the prevention of mother to child transmission program (United Nations Children Fund (UNICEF), 2012).

In Uganda, HIV/AIDS remains a significant public health problem; roughly 1,400,000 people were living with HIV/AIDS in 2011, including 190,000 children less than 15 years, and there were about 64,000 deaths due to AIDS during the same time period (Uganda AIDS Commission, 2012; UNAIDS, 2012). The prevalence of HIV among the children less than 5 years old was about 0.7% in 2011 (Ministry of Health & ICF International, 2012; UNAIDS, 2012). Whereas the country had succeeded in reducing the prevalence of HIV from about 30% in 1990 to 6% in 2005, an increase in prevalence has been experienced in the last 6 years due to the higher survival rate of HIV-infected people as a result of increased access to HAART. The higher prevalence is also contributed to by the increasing number of new HIV infections due to complacency in implementation of preventive measures (Ministry of Health, Uganda, ICF International, Centers for Disease Control and Prevention Entebbe, Uganda, U.S. Agency for International Development Kampala, Uganda, WHO Uganda Kampala, Uganda, 2008). About 40% of the 1.4 million people living with HIV were eligible for HAART in

2011 based on the contemporary WHO guidelines. The WHO guidelines recommended initiating HAART when immunity had reduced to a level where CD4+ count, a measure of immunity level, is 350/ml. However, only about 266,236 adults and 25,000 children less than 14 years old were on HAART in 2011, comprising 54% and 31% of the adults and children respectively that need HAART (Uganda AIDS Commission, 2012).

Effect of HIV Infection on Measles Vaccine Effectiveness and Control

The effect of HIV infection on the production of protective antibodies following measles vaccination in Uganda was first studied in 1999 by Waibale, Bowlin, Mortimer, and Whalen. Their findings indicated that the measles antibody response among well-nourished HIV-infected and HIV-uninfected children was similar. The authors concluded that it is not HIV infection per se but the malnutrition among HIV-infected children that affects the antibody response to measles vaccination. In another study conducted in Malawi by Fowlkes et al. (2011), a lower proportion of HIV-infected children, compared to HIV-uninfected children, mounted protective levels of measles antibody. Fowlkes et al. (2011) found that antibodies waned gradually among HIV-infected children and by 20 months only 40% of these children had protective level of measles antibodies, compared to 84% of HIV-uninfected children.

Furthermore, a study conducted in Central African Republic by Tejiokem et al. (2007) also found that measles antibodies waned among HIV-infected and HIV-exposed uninfected children aged 18 to 36 months. Only 20% and 56 % of HIV-infected and HIV-exposed but uninfected children respectively still had protective measles antibodies in this age group. However, Fowlkes at al. (2011) did not assess for a possible relationship

between nutrition status and the waning of antibodies, and Tejiokem et al. (2007) found malnutrition not significantly associated with unprotective measles antibodies. In their study in Uganda, Kizito et al. (2013) found malaria, HIV infections during pregnancy and in infants, and malnutrition as significant factors contributing to a reduction of the measles antibody response, implying that both HIV infection and malnutrition independently contribute to reduced vaccine effectiveness.

The death rate among HIV-infected children was high in developing countries in the pre-HAART era, with over 50% dying by 2nd year and about 62% by 5th year (Brahmbhatt et al., 2006; Obimbo et al., 2004). As a result, HIV-infected children were not a large threat to measles control (Helfand et al., 2005). However, the waning antibodies among HIV-infected children yet with increased survival of the children on HAART may result in a pool of children susceptible to measles who could maintain measles transmission in high HIV-prevalence settings (deMoraes-Pinto, 2011; Moss & Grant, 2012; Moss & Strebel, 2011). The waning of antibodies among HIV-infected children could result in secondary vaccine failure that may predispose vaccinated HIV-infected children to higher incidence of measles (Biellik, 2010). The initial success in measles control efforts in South Africa, a country hyperendemic for HIV, with 460,000 children less than 14 years old living with HIV (UNAIDS, 2012), created the impression that HIV infection may not be a big threat for measles control and elimination (Bellini & Rota, 2011; Helfand et al., 2005). Yet, in a study by Sartorius et al. (2013), the authors indicated that HIV infection was one of the significant contributing factors to sporadic measles outbreaks in South Africa. Other factors included failure to vaccinate and high

population density. It is thus uncertain if the high HIV prevalence in the African region may affect the measles elimination timelines set by the region.

There is very limited documentation on the effect of HIV infection on response to the measles vaccine and on measles illness in Uganda (Kizito et al., 2013; Korutaro, Tukei, Baruga, Asiimwe, & Kekitiinwa, 2013; Waibale et al., 1999). Uganda has a high HIV-prevalence of 8.3% among women of the reproductive age group and there are increasing new infections especially among this group (Ministry of Health & ICF International, 2012). About 100,000 HIV-infected pregnant women are seen and over 20,000 HIV-infected children born annually (UNICEF, 2012). With increasing access of the HIV-infected children to HAART, HIV infection could be a threat for measles control in Uganda. Additionally, with the higher survival rates among children on HAART in Uganda, secondary vaccine failure could produce a large pool of susceptible children and young adults who can maintain measles virus transmission even when very high measles coverage of over 95% is attained. Children with secondary vaccine failure may also be at higher risk for measles morbidity and mortality. There is a need for further studies on the effect of HIV infection and HAART on measles morbidity and mortality in Uganda. Results of these studies could inform the national approach to measles control for HIV-infected children on HAART in Uganda and other Sub-Saharan African countries, where most HIV-infected children live (UNAIDS, 2012). Specifically, the results could generate evidence for a vaccination policy for HIV-infected children on HAART. Such a policy could ensure that these children are not susceptible to measles, avoiding accumulation of

a pool of measles susceptible children in the community. This would enhance attainment of the measles elimination in the country.

Statement of the Problem

Measles remains a major cause of morbidity and mortality globally (WHO, 2009a). In spite of availability of an effective vaccine and significant progress in measles control achieved in the last decade, the disease still caused 158,000 deaths globally in 2011 (WHO, 2013a, 2013b, 2013c). In Uganda, efforts aiming at accelerated measles control resulted in reduction of measles morbidity and mortality significantly, by 93% and over 95% respectively, in 2006 compared to 2000 levels (Mbabazi et al., 2009). Measles morbidity and mortality was subsequently maintained at very low levels between 2007 and 2009. However, following this success and continued efforts to maintain high measles vaccination coverage in Uganda, there was an upsurge in measles cases and deaths from 2010 to 2013 (Measles Surveillance and National Health Management Information System databases). Factors that contributed to the upsurge of measles in Uganda were not fully investigated but could include lower vaccine effectiveness, low vaccine coverage, and unknown risk factors among a population with a high HIV prevalence (Ministry of Health Uganda, 2013; Ministry of Health & ICF International, 2012).

Given the primary and secondary vaccine failure experienced by HIV-infected children (Biellik, 2010; Fowlkes et al., 2011; Kizito et al., 2013; Tejiokem et al., 2007), the longer survival of HIV-infected children on HAART could have produced a pool of measles susceptible children in Uganda that contributed to the upsurge of measles.

Additionally, measles antibodies in vaccinated HIV-infected children wane and by 2 – 3 years of age, 60% to 80% of children have secondary vaccine failure (Fowlkes et al., 2011; Tejiokem et al., 2007). In measles endemic and high HIV-prevalence areas like Uganda, this could imply that HIV-infected children on HAART older than 2 years may have higher measles susceptibility than HIV-uninfected children in the same age group. However, there is a gap in information on whether there is an increase in the risk of measles among HIV-infected children on HAART, and whether there are factors that could predict the risk of measles in these children. Additionally, it is not yet clear what would be the most appropriate age for revaccination of HIV positive children on HAART (Bellini & Rota, 2011; Moss & Griffin, 2012). There are still gaps and inconsistent information on the effect of HIV infection on measles susceptibility, with some researchers suggesting no contribution and yet others indicating that HIV infection is a contributing factor to upsurges in measles and outbreaks (Sartorius, 2013). Therefore, more studies are needed to elucidate further the risk of measles and assess predisposing factors among HIV-infected children older than 2 years on HAART to guide measles control in this group.

Purpose of the Study

The purpose of this quantitative study was to examine whether there is a difference between the risk of measles in HIV-infected children on HAART and that of HIV-uninfected children of the same age group. I also examined the relationship between selected risk factors (malnutrition, age at initiation of HAART, and low CD4+ count) and the risk of measles in HIV-infected children on HAART. Additionally, I ascertained the

age group in which HIV-infected children on HAART were most affected by measles, in order to generate information that could guide decision on the appropriate age for potential revaccination.

Research Questions and Hypotheses

This study sought to answer the following research questions:

Primary Research Question

PQ. Is there a difference in the risk of measles among HIV-infected children on HAART compared to uninfected children of the same age group?

$H_0 1$: There is no difference between the risk of measles among HIV-infected children on HAART and the risk of measles among HIV-uninfected children of the same age group.

$H_a 1$: There is a difference between the risk of measles in HIV-infected children on HAART and the risk of measles among HIV-uninfected children of the same age group.

Secondary Research Questions

RQ1. What is the age group most affected by measles among the HIV-infected children on HAART?

RQ2. Are age at initiation of HAART, low nutritional status (stunting, wasting, and underweight), and low CD4+ count prior to measles illness or outbreak (CD4+ percentage of less than 25%) significant predictors of measles in HIV-infected children on HAART?

$H_0$2: Age at initiation of HAART is not a significant predictor of measles in HIV-infected children on HAART.

H_a2: Age at initiation of HAART is a significant predictor of measles in HIV-infected children on HAART.

$H_0$3: Nutritional status (undernutrition) is not a significant predictor of measles in HIV-infected children on HAART.

H_a3: Nutritional status (undernutrition) is a significant predictor of measles in HIV-infected children on HAART.

$H_0$4: Low CD4+ count is not a significant predictor of measles in HIV-infected children on HAART.

H_a4: Low CD4+ count is a significant predictor of measles in HIV-infected children on HAART.

Theoretical Base

The proximate determinants framework (Boerma & Weir, 2005) was the conceptual framework used to guide the study. The proximate determinants framework was conceptualized slightly over 50 years ago by Davis and Blake (Boerma & Weir, 2005) as an analytical framework for comparative studies on fertility. It was further developed by Bongaarts in 1978 into the proximate determinants framework that has been frequently used in studies on fertility (Boerma & Weir, 2005). It was adapted by Mosley and Chen in 1984 into a conceptual framework for child survival in developing countries, and by Boerma and Weir (2005) into the proximate determinants framework for guiding studies on the determinants of HIV infection (Boerma & Weir, 2005). The

principle behind the proximate determinants framework is that there are several interlinked factors at different levels of influence that contribute to the good or ill health of a population.

Some of the factors are further removed from a health condition but contribute to the health condition indirectly (underlying determinants) through their influence on factors closely linked to or near the health condition (proximate or intermediate determinants). The proximate determinants in turn influence some biological changes within the body (immediate determinants) that directly lead to or cause the health condition (Boerma & Weir, 2005; Krieger, 2008; Mosley & Chen, 1984).

The underlying determinants could be related to the context (e.g. demographic features, socioeconomic and cultural factors, as well as disease endemicity), or could be related to the ongoing health or non-health interventions in the community (Boerma & Weir, 2005; Krieger, 2008; Mosley & Chen, 1984). These underlying factors influence changes in the proximate factors that are behavioral or biological in nature, for example attitudes, beliefs, and genetic changes. The proximate determinants in turn influence the immediate determinants that are biological in nature, such as immunity level (low CD4+ count or antibody levels), exposure to infectious people, and duration of infectivity. The immediate determinants precipitate or result in ill health or undesirable health outcome.

The proximate determinants framework thus depicts the complex nature of ill health causation and causation pathways (Mosley & Chen, 1984; Boerma & Weir, 2005; Krieger, 2008). It also depicts the ways in which ill health could be caused by linkages between different underlying, proximate, and immediate determinants and provides a

framework for research into the possible contributors to or causes of ill health (Boerma & Weir, 2005). Researchers can study the interactions of different determinants and their effect on ill-health using this conceptual framework. The framework can also be used to design different interventions for the different levels of determinants. The proximate determinants framework thus highlights the importance of underlying factors that influence proximate factors; the proximate factors then influence the immediate or biological factors that result in infection, morbidity, and mortality (Boerma & Weir, 2005).

The proximate determinants framework was considered appropriate for this study given that the risk of measles in HIV-infected children on HAART is likely to be influenced by several linked factors. Some of the factors are immediate, others proximate, while others are underlying factors. The framework could also be used to guide design of interventions to address the different factors to reduce the risk of measles for these children. The framework was adapted for this study, particularly to include the determinants at the different levels that influence or are likely to influence the occurrence of measles in HIV-infected children on HAART. The proximate determinants framework and adaptations made are discussed in more detail in Chapter 2.

Nature of the Study

This research was a quantitative retrospective cohort study. From a cohort of children 2 to 15 years living in the communities in Kampala and Wakiso districts, a sample of 223 HIV-infected children on HAART (exposed children) and 223 HIV-uninfected children (unexposed) was selected. The sample was selected from the cohort

members that attended Nsambya Hospital Home Care Department (HCD) in Kampala, a department that provides comprehensive care for HIV-infected children and adults, and Nsambya Hospital Out-Patient Department (OPD) in the period from November 2011 to June 2012, when there was a measles epidemic in Kampala and Wakiso districts.

Data for both exposed and unexposed children were collected from their medical records and entered into a data collection tool. Data collected included demographic details, vaccination history, and measles illness during the measles outbreak in November 2011 to June 2012, or thereafter. If measles illness was reported in the records, the date and age when illness was developed, clinical presentation, and any complications developed were recorded. Data were also collected on other variables that could be potential confounders, effect modifiers, or predictors of the relationship between HIV infection and measles illness among HIV-infected children on HAART. These variables included nutrition status before measles illness or outbreak for HIV-infected children on HAART (using weight for age, height for age, and weight for height), age, CD4+ count and percentage at HAART initiation, and latest CD4+ count and percentage prior to measles illness or outbreak.

Weight for age, height for age, and weight for height (or body mass index [BMI] for age) measure different forms of malnutrition. Height for age is a measure of linear growth and can be used to assess chronic malnutrition (stunting) in children (Shetty, P., n.d.). Weight for age is a measure of growth in relation to chronological age and the children with undernutrition for weight for age are underweight (Shetty, P., n.d). Weight for height (or BMI for age) is a measure of the weight of an individual in relation to

his/her height and indicates whether an individual is wasted or not (Shetty, P., n.d). Thus, children with undernutrition for weight for height or BMI for age are wasted.

The terms stunting, underweight, and wasting were used in this study to refer to the three different types of malnutrition. Stunting is an indication of chronic malnutrition while wasting is an indication of recent or acute malnutrition (Shetty, n.d). Being underweight is an indication of disharmony between the linear growth and body composition in reference to age (Shetty, n.d). Each of these types of malnutrition could affect the risk of measles differently in HIV-infected children. Stunting, an indication of chronic malnutrition, can result in primary or secondary measles vaccine failure, and could thus increase the risk of measles (Waibale et al., 1999). Wasting in a child is an indication of acute malnutrition and is associated with primary and possibly secondary vaccine failure and high mortality among children with measles, especially in the presence of vitamin A deficiency (Franca et al., 2009; Katona & Katona-Apte, 2008; Kizito et al., 2013). Underweight status may also increase the risk of measles illness and mortality (Korutalo et al., 2013).

As these variables could be potential confounders, effect modifiers, or predictors for the risk of measles, analysis was done to assess and control for their effect. For the primary question, the dependent variable was measles illness, while the independent variable was HIV infection on HAART. Age, sex, and vaccination status were covariates. In secondary question two, the dependent variable was measles illness while the independent variables were age at initiation of HAART, under nutrition (stunting, wasting, and underweight), and CD4+ prior to exposure to measles outbreak. Age, sex,

duration on HAART, and CD4+ prior to initiation of HAART were covariates.

Furthermore, as the children were of different ages within the target age group, the study

provided information on risk of measles at different ages among children who are on

HAART as well as uninfected children attending OPD. More details on the methodology

of the study are provided in Chapter 3.

Definition of Terms

Herd immunity: This term is used to refer to a situation whereby a high proportion of

individuals in a community are immune to an infection such that this offers protection

against the infection to non-immune individuals. Thus the risk of infection among the

non-immune individuals (susceptibles) in a community is reduced by the presence and

proximity of high numbers of immune individuals, resulting in reduced incidence of the

infection in the community (Fine, Eames, & Heyman, 2011).

HIV-exposed but uninfected: This refers to children born to HIV-positive mothers and

hence exposed to HIV but who do not have HIV infection as evidenced by a negative

virological test for HIV or its components (HIVRNA or HIVDNA or ultrasensitive HIV

p24 antigen) (WHO, 2007).

HIV-infected: Among adults and children 18 months or older, an HIV-infected person is

defined as one with:

    • Positive HIV antibody testing (using rapid or laboratory based enzyme linked

    immunoassay). This is further confirmed by a second HIV antibody test (rapid or

    laboratory based enzyme linked immunoassay) using different HIV antigens or

    different operating procedures (WHO, 2007);

and/or;

 • Positive virological test for HIV or its components (HIVRNA or HIVDNA or

ultrasensitive HIV p24 antigen) confirmed using another virological test with

different operational procedures (WHO, 2007).

Among children younger than 18 months, an HIV-infected child is defined as one with:

 • Positive virological test for HIV or its components (HIVRNA or HIVDNA or

ultrasensitive HIV p24 antigen) confirmed by a second virological test using another

specimen collected more than four weeks after the first one (WHO, 2007).

Immune recovery: Defined as CD4+ percentage of greater than 15% for three consecutive

months in an HIV-infected person on HAART whose CD4+ percentage was initially

lower than this (Aurpibul, Puthanakit, Sirisanthana, & Sirisanthana, 2010).

Immediate determinants: Refers to biological processes or situational events that

precipitate or result in ill-health or other undesirable outcomes (Boerma & Weir, 2005).

Low CD4+: Defined as CD4+ count less than 25% of total lymphocytes (Panel on

Antiretroviral Therapy and Medical Management of HIV-infected Children, 2014)

Measles susceptibility: Prone to measles infection evidenced by absence of protective

antibodies to measles or when the level of measles antibodies is below the level

considered to be protective, which is less than 120mIU (Bellini & Rota, 2011).

Primary vaccine failure: This refers to when vaccinated individuals fail to mount

protective antibodies in response to their first dose of vaccine (Ramsay & Brown, 2013).

Proximate determinants: Are factors, behavioral or biological in nature, with more direct effects on biological processes or events that precipitate ill health or other undesirable outcomes (Boerma & Weir, 2005).

Secondary vaccine failure: This occurs when vaccinated individuals develop protective antibodies after vaccination but lose the protective antibodies over time (Ramsay & Brown, 2013).

Assumptions

The study participants were limited to children in Wakiso and Kampala only, where there was evidence of measles transmission, with a big outbreak in the districts from November 2011 to June 2012. It was thus assumed that study participants were all exposed to the measles virus during the outbreak period. It was also assumed that children who attended the HCD and OPD at Nsambya hospital, the study site, during the measles outbreak were representative of the cohort of children in Kampala and Wakiso then, as they resided within the different communities in the districts. The CD4+ count, weight and height measurements at the last follow up visit preceding a measles episode or the measles outbreak in HIV-infected children on HAART were assumed to be the CD4+ level, weight and height at time of measles illness or outbreak since CD4+ levels and these parameters are only monitored every 1 to 3 months. Children with no indication in their records of being HIV positive and or who were indicated as HIV negative in their medical records were assumed to be HIV negative and thus considered unexposed. This was based on the practice in Nsambya hospital where HIV positive children are actively looked for through early infant diagnosis and regular HIV counseling and testing in the

hospital catchment area to initiate them into HIV comprehensive care early in life

(Massavon et al., 2013).

Limitations

One of the limitations in this study was the lack of or incomplete data in some of

the medical records given that data were retrieved from past records. This applied to data

on some variables including history of vaccination, CD4+ count and percentage at

initiation of HAART, CD4+ count/percentage, weight and height prior to measles illness

and outbreak. In addition, there was a possibility of inaccurate classification of

unexposed children if for some reason HIV positive status of a child was not indicated in

his/her medical records. Such a child would be inaccurately classified as unexposed.

Delimitations

In this study, I enrolled participants from only Kampala and Wakiso districts.

These districts experienced a measles outbreak from November 2011 to June 2012 and

hence children from these districts were considered to have been exposed to measles

virus during this period. Measles antibodies in HIV-infected children have been found to

wane quickly resulting in secondary vaccine failure such that by 2 to 3 years of age only

about 20% of HIV-infected children still have post vaccination protective levels of

measles antibodies (Fowlkes et al., 2011; Tejiokem et al., 2007). This implies that most

HIV-infected children 2 years old and above could be susceptible to measles. In this

study, I thus enrolled exposed and unexposed children that were between 2 and 15 years

of age at time of exposure to measles virus in the measles outbreak of November 2011 to

June 2012. The study participants were recruited from Nsambya Hospital HCD and OPD

and it was assumed that these children were representative of the cohort 2 to 15 years in Kampala and Wakiso districts at the time of measles outbreak, from November 2011 to June 2012.

Significance

Measles is a highly infectious disease. It is a major cause of morbidity and mortality globally. About 355,000 cases of measles and 158,000 deaths due the disease were reported globally in 2011 (WHO, 2013a, 2013c). Efforts are ongoing in all endemic countries including Uganda to increase measles vaccination coverage to over 95% to build high immunity level at community levels, and limit the number of children susceptible to measles to a minimum number that cannot maintain transmission of measles (WHO, 2012). However, HIV infection is a factor that could potentially hinder attainment of high levels of immunity to measles, a requisite to prevent measles outbreaks among vaccinated children, in Uganda due to primary and secondary vaccine failure (Kizito et al., 2013; Moss & Strebel, 2011; Uzicanin & Zimmerman, 2011). HIV infection could thus result in development of a pool of susceptible children that could maintain measles transmission in a high HIV-prevalence setting like Uganda. (Moss & Griffin, 2012; Moss & Strebel, 2011). Susceptible children are likely to accumulate faster and in large numbers as more HIV-infected children access HAART and survive into adulthood.

In this study, I examined the risk of measles among HIV-infected children on HAART compared to HIV-uninfected peers, and the age group of highest susceptibility to measles in the HIV-infected children on HAART, and hence the possible timing for

revaccination of the HIV-infected children. Additionally I examined factors that were associated with a higher risk of measles in HIV-infected children on HAART in Uganda. The findings of this study contribute to better understanding about the risk of measles in HIV-infected children on HAART and could inform policy and practice changes pertaining to measles vaccination among HIV-infected children on HAART in Uganda, and possibly within Sub-Saharan Africa. HIV-infected children tend to develop severe measles illness and have higher mortality rates from measles than the HIV-uninfected children (deMoraes-Pinto, 2011). The study findings will be used to guide vaccination strategies in HIV-infected children in Uganda, which could contribute to reduction of measles morbidity and mortality among HIV-infected children, resulting in positive social change especially for this group of children and their families. Furthermore, the study findings could contribute to measles elimination efforts by providing new information on how to reduce the number of children susceptible to measles among HIV-infected children. The reduction of children susceptible to measles will in turn produce positive social change in communities that have suffered from measles morbidity and mortality for ages. The findings of the study could also provide critical information to ongoing global discussions among policy makers and measles experts on the effect of HIV infection on measles control and elimination and the need for booster doses of measles vaccine for HIV-infected children on HAART. The study findings could thus specifically inform and influence measles vaccination practice and policy for HIV-infected children, and particularly those on HAART in Uganda.

Summary

Measles remains a major public health problem globally, however, there are ongoing efforts to eliminate the disease by 2020 (Goodson et al., 2012; WHO, 2011). Attainment and maintenance of high population immunity to measles (herd immunity) is necessary for elimination to be achieved (Moss & Strebel, 2011). Several programmatic and host factors cause primary or secondary measles vaccine failure hence affecting the vaccine effectiveness (Kizito et al., 2013; Moss & Strebel, 2011; Uzicanin & Zimmerman, 2011). Infection with HIV has been shown to result in fast decline of measles antibodies following measles vaccination; such that by 2 years most vaccinated HIV-infected children do not have protective levels of measles antibody (Fowlkes at al., 2011; Tejiokem et al., 2007). These children are thus susceptible to measles and could maintain measles transmission and hamper measles control and elimination efforts (Moss & Griffin, 2012; Moss & Strebel, 2011).

There has been an upsurge of measles in Uganda since 2010, with outbreaks in different districts, involving both children less than 5 years of age, older children and adults (Ministry of Health, 2013). However, it is not clear whether secondary vaccine failure due to HIV infection is contributing to the high numbers of susceptible children and measles cases. It is also not clear whether initiating HAART early before the deterioration of the immune system could prevent or modify the rate of waning of measles antibodies in the HIV-infected children, or whether the HIV-infected children on HAART older than 2 years, experience a higher incidence of measles than HIV-uninfected children in the same age group. There is also inadequate information on the

benefits of measles revaccination and appropriate age of revaccination among these children.

In this quantitative research, I used a retrospective cohort study design to examine the risk of measles among HIV-infected children on HAART compared to their HIV-uninfected peers. In addition, the age group of highest susceptibility to measles in the HIV-infected children on HAART and hence the possible timing for revaccination, and the possible role of age at initiation of HAART, low CD4+ count, and undernutrition as predictors of the risk of measles in the HIV children on HAART were analyzed. The study site was Nsambya hospital, in Kampala, Uganda. From the cohort of 2 to 15 years old in Kampala and Wakiso districts in November 2011 and June 2012, exposed and unexposed children were selected from the cohort members that attended Nsambya hospital HCD and OPD from November 2011 to June 2012. Exposed children were HIV-infected children on HAART, 2 to 15 years old, while unexposed children were HIV-uninfected children of the same age group. The proximate determinants framework was the conceptual framework guiding the study. The findings of this study could inform policy and practice pertaining to measles vaccination among HIV-infected children in Uganda, and possibly, within Sub-Saharan Africa, which could contribute to reduction of measles morbidity and mortality among HIV-infected children. A detailed review of literature related to the study is included in Chapter 2, while Chapter 3 covers the detailed methodology of the study.

Chapter 2: Literature Review

Introduction

Secondary measles vaccine failure in HIV-infected children 2 years and older could result in HIV-infected children on HAART, who survive much longer than 2 years, being more susceptible to measles than their HIV-uninfected peers. The purpose of this study was to examine the risk of measles among HIV-infected children on HAART compared to their HIV-uninfected peers. In addition, I analyzed the age group of highest susceptibility to measles in the HIV-infected children on HAART, and the possible role of age at initiation of HAART, low CD4+ count, and undernutrition as predictors of the risk of measles in the HIV-infected children on HAART.

In this chapter, I describe the search strategy that I used to generate the literature for review in the study, and critically review the literature on measles and related risk factors in HIV-infected children on HAART. Additionally, I also include a critical review of literature on the relevance and utility of measles revaccination of HIV-infected children on HAART. Gaps that my study tried to address are highlighted. The chapter also includes a detailed review of the proximate determinants framework on which this study was based.

Literature Search Strategy

I conducted the literature search using Google and Google Scholar search engines as well as the Academic Search Complete and PUBMED+CIANHL databases from the Walden University Library. In Google, different phrases were used to search for literature of interest and out of the literature generated, peer reviewed literature published in the

last 10 years were selected. Some peer-reviewed literature that had special information of interest to the study but was published over 10 years ago was also selected.

Additionally, literature from specialized agencies including the WHO, CDC, UNAIDS, UNICEF, and UBOS were selected based on their relevance to the topic of study. The phrases used in Google search included: *risk of measles in HIV-infected children, HIV and measles in Uganda, immune response to measles in HIV-infected children,* and *measles and HIV infection*. Some articles of interest were accessed through Google or Google Scholar directly if they were open access or a free full text article was available; however, for some articles the authors, titles, and publication years were noted and used to search for these articles through the Walden University Library. When articles were accessed in Google and Google Scholar, the list of related articles was reviewed and whenever a relevant article was found from the list of related articles, the article was downloaded.

I used the Walden University Library, Academic Search Complete, Google Scholar, PubMed, and CINAHL &Medline Simultaneous Search to search for relevant literature. The terms used in searching for literature included*: measles and HIV infection and HAART, measles and vaccine response and HIV infection, measles immunization and HIV and Africa, measles immunization and HIV and Uganda, measles epidemic, HIV infection, Africa, HIV infection and HAART* and *revaccination.* Search phrases in text and peer reviewed articles from 2003 to 2015 were utilized to guide the search. Relevant articles identified were downloaded and saved in a folder. In a few cases, only abstracts were found and not full text articles. I read the abstracts and, in one example in the

literature review, used only the information provided in the abstract. In this chapter, I

present more details on the proximate determinant framework, and review the literature

generated on measles, HIV and AIDS, measles antibodies in HIV-infected children. I also

critically review the literature on the role of HAART in rebuilding the immunity, the risk

of measles in HIV-infected children on HAART and the possible predictors, and

revaccination of HIV-infected children on HAART.

Proximate Determinant Framework

The proximate determinant framework examines the complex nature of disease

causation and identifies indirect and direct determinants of disease and mortality (Boerma

& Weir, 2005). The proximate determinants framework has been used in designing

studies and interpreting study results on fertility, maternal mortality, child mortality, and

HIV incidence and prevalence. The framework is adaptable to study different infectious

disease conditions (Boerma & Weir, 2005). The framework was adapted for this study,

particularly to include the determinants at the different levels that influence or are likely

to influence the occurrence of measles in HIV-infected children on HAART. In the

proximate determinants framework by Boerma and Weir (2005), the underlying

determinants include context factors such as socioeconomic, sociocultural, and

demographic factors. In the adaptation of the framework for this study, measles

endemicity was added as an important underlying factor in the context. Under the

interventions, more programs were added including vaccination, nutrition education and

care, and a few HIV interventions that are linked to HAART. In the proximate

determinants framework by Boerma and Weir (2005), the proximate determinants include

treatment with HAART and opportunistic infections, as well as several behavioral and biological factors. In the adapted framework for this study, HIV infection and malnutrition were included in the proximate factors as well. The immediate determinants were also adapted to include low CD4+ percentage, primary and secondary vaccine failure, and survival of HIV-infected children. The adapted framework depicts possible pathways for measles occurrence among HIV-infected children on HAART. The underlying contextual factors can influence the level of HIV infection and indirectly influence the level of exposure of susceptible children to measles infection in a community.

The intervention programs identify children with HIV infection and their treatment for opportunistic infections and with HAART. These programs also identify of children with malnutrition. HIV infection and malnutrition can influence the level of immunity (CD4+ count), although among the HIV-infected children, the immunity level can be modified by treatment with HAART and treatment for opportunistic infections. HIV infection can also result in primary and or secondary vaccine failure that could possibly increase the susceptibility of HIV-infected children to measles, in a context of measles endemicity.

Treatment with HAART contributes to longer survival of HIV-infected children, which could result in increased susceptibility to measles given the possibility of primary and secondary vaccine failure.. In the context of measles endemicity, HIV-infected children on HAART, who are possibly susceptible to measles due to primary or

secondary vaccine failure could be exposed to people with measles infection, be infected, and keep measles transmission in the community.

In line with the adapted framework (Figure 1), in this study I examined whether HIV infection and treatment with HAART contribute to higher susceptibility to and risk of measles in HIV-infected children on HAART compared to HIV-uninfected children of the same age group. The role of malnutrition as a possible proximate determinant was also analyzed. In addition, the possible role of low CD4+ count/percentage as an immediate determinant of measles in HIV-infected children was analyzed. As my study findings showed higher risk of measles among HIV-infected children on HAART, though not statistically significant, HIV infection with HAART treatment could be a proximate determinant of measles in Uganda.

Background or underlying determinants, including social cultural context, influence occurrence of disease. They influence uptake of disease prevention and control interventions in communities (Berkman & Kawachi, 2000; Emmons, 2000). For example, sociocultural practices in some sub-Saharan African countries have in the past contributed to high rates of malnutrition in some communities (Luchuo et al., 2013). Similarly, the socioeconomic and sociocultural factors in Africa in general and Uganda in particular have contributed to a high prevalence of HIV in the country and in the region (Asiimwe, Kibombo, & Neema, 2003). These underlying determinants are generally "far" from the disease conditions and the biological processes but tend to influence indirectly the occurrence of diseases by creating an environment that either enhances or prevents disease (Boerma & Weir, 2005; Krieger, 2008). Changes in the underlying determinants

influence a set of variables, called "proximate determinants," that are closer to the disease

conditions (Boerma & Weir, 2005; Krieger, 2008).

Changes in the proximate determinants, which are biological and or behavioral in

nature, have a more direct influence on immediate determinants of disease, and thus a

more direct effect on the demographic outcome (Boerma & Weir, 2005). For example,

HIV infection, a proximate determinant in the proximate determinants framework, has a

direct effect on the B and T cell functions resulting in deterioration of individuals'

immunity (Pensieroso et al., 2009). The reduced immunity is an immediate determinant

of ill-health, possibly including measles illness. HIV infection has also been shown to

cause decline in measles antibody levels to below protective level hence causing measles

vaccine secondary failure (Aurpibul et al., 2006; Fowlkes et al., 2011; Sutcliffe & Moss,

2010; Tejiokem et al., 2007). The secondary vaccine failure is an immediate determinant

of measles susceptibility and could hence directly influence occurrence of measles.

Since the proximate determinants framework displays a web of the different

possible pathways of disease or ill health causation, it is useful in the design of studies

and effective interventions, in the analysis of risk factors and interpretation of results of

intervention studies, and in ecological studies. Studies are designed to investigate

different causation pathways, relationships between determinants at different levels and

disease occurrence, or between determinants at different levels. In this study, the

relationship between HIV infection and HAART (possible proximate determinants) and

measles disease, and between CD4+ percentage (immediate determinant) and measles

disease were examined. In addition, the relationship between nutrition status of HIV-infected children (possible proximate determinant) and measles illness were examined.

Krieger (2008) argued that the proximate determinant and similar frameworks that use a distal-proximate approach depict disease causation pathways as linear or sequential and yet they are more complex. Krieger (2008) further argued that underlying or distal determinants can directly influence disease causation and distribution without going through the proximate determinants. Furthermore, it is argued that the proximate determinant framework and other similar frameworks do not take into consideration class and racial inequalities, political power, and ecology, all of which contribute to the distribution of disease, and do not consider the causal pathways at multiple levels (Krieger, 2008).

Krieger (2008) also asserts that the proximate determinant and other similar frameworks result in more focus on proximate factors that are amenable to control by individuals or public health and less focus on the underlying or distal determinants that require societal interventions and changes, which may hinder multilevel thinking about causation. However, the proximate determinant framework clearly indicates that contextual factors including social, economic, cultural, political, and other environmental factors contribute to disease causation. Each component of the underlying determinants could be expanded further in studying disease causation pathways to include class and racial inequalities as well as politics and power where relevant. While the proximate determinant framework is useful in understanding disease causation, it is also very useful in designing interventions for prevention or control of the disease conditions (Boerma &

Weir, 2005). Interventions should be designed to address the underlying, proximate, and immediate determinants and not only the proximate determinants (Berkman & Kawachi, 2000; Emmons, 2000). The proximate determinants framework presents in a systematic manner the possible multifactorial contributions to ill-health at the population level (Boerma & Weir, 2005) and does not hinder examination of causes at other levels if necessary.

The different arrows in Figure 1 show that different underlying factors could affect different proximate and immediate factors and hence the framework does not rule out underlying factors having direct influence on the disease outcome nor does it emphasize one sequence of events. The proximate determinants framework thus remains a useful tool for understanding disease causation pathways, guiding research, and designing interventions (Boerma & Weir, 2005). It was highly relevant for my study on the risk factors for measles in HIV-infected children on HAART.
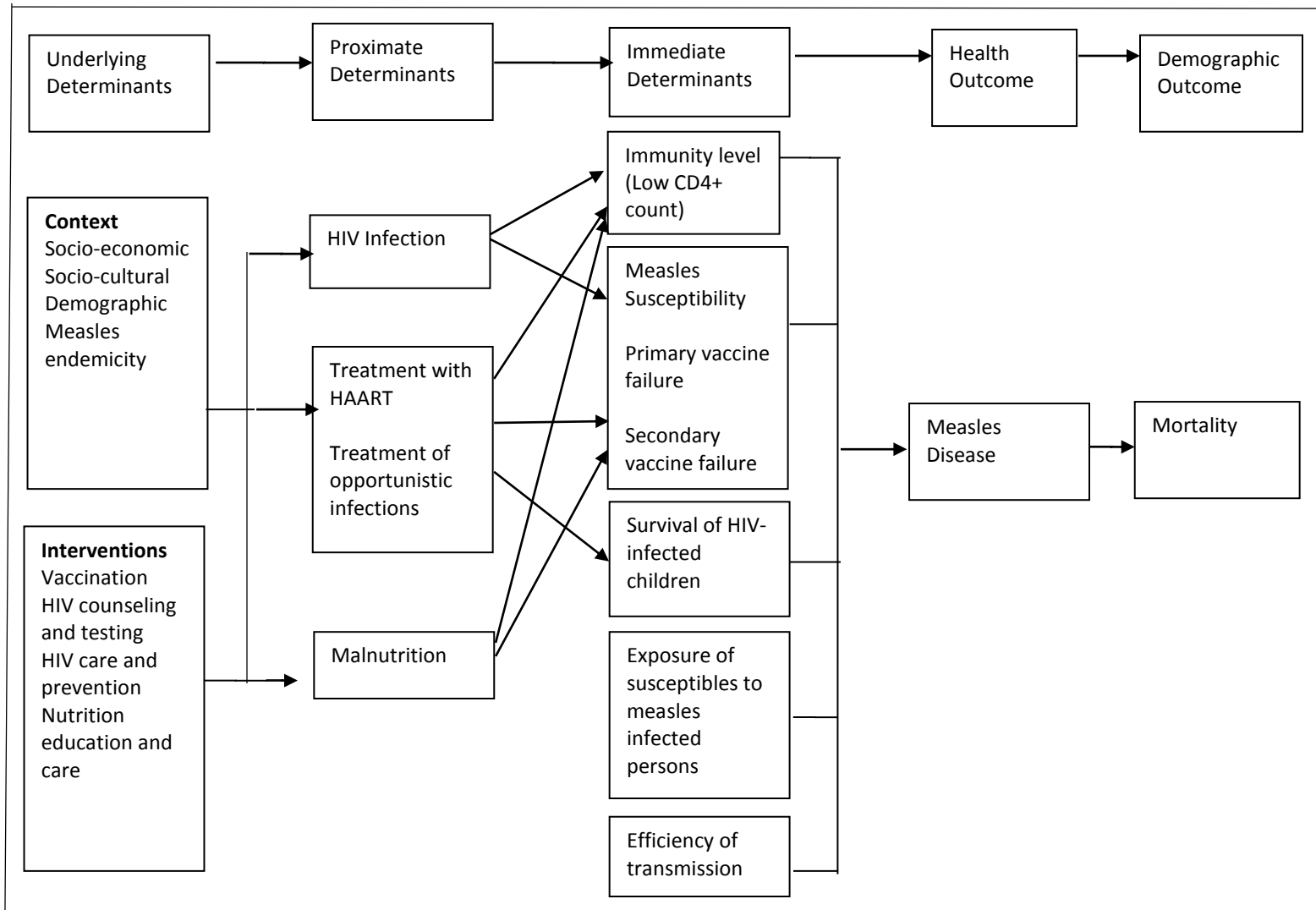
Figure 1. Proximate determinants framework for risk factors for measles in HIV-infected children in Uganda

Measles

Measles is an illness caused by infection with the wild measles virus, transmitted by aerosolized respiratory droplets from infected persons. Measles is a highly contagious disease and affects over 90% of individuals not vaccinated against it in an affected community (WHO, 2009a). The incubation period for measles is 10 to 14 days (WHO, 2009a). A measles infected person typically presents with fever, with temperature above 38oC, and a maculo-papular rash on the whole body. Other symptoms include red eyes (conjunctivitis), cough, running nose (coryza), and sores in the mouth. Koplik spots, bluish white patches seen in the mouth, are clinical diagnostic features (pathognomonic) of measles (Moss & Griffin, 2012; WHO, 2009a).

The disease may be self-limiting but often causes severe morbidity, disability, and death due to complications including pneumonia, otitis media, malnutrition, laryngo-tracheobronchitis, cornea opacities causing blindness, measles encephalitis, and subacute sclerosing panencephalitis (Moss & Griffin, 2012; WHO, 2009a). Severe measles disease tends to occur in children less than 5 years, children with malnutrition, and children with immunosuppression due to different causes including HIV infection (WHO, 2009a). It is a common cause of morbidity and mortality in measles endemic communities in developing countries with weak health systems especially in children less than 5 years (WHO, 2009a).

Measles is prevented by infant vaccination with measles vaccine (Moss & Griffin, 2009; WHO, 2009a, 2009b). Mothers who were vaccinated or exposed to wild measles virus transfer maternal measles antibodies to their newborns at birth. The

maternal antibodies in the infants protect them against measles in the first 6 to 9 months of life, however the antibodies gradually wane and by 9 months are quite low or nonexistent in most of the infants (WHO, 2009a, 2009b). Measles vaccination is part of the routine immunization services in all countries. In most endemic countries, it is scheduled at 9 months when maternal antibodies are expected to have waned (WHO, 2009a, 2009b). When still present in high levels, maternal measles antibodies interfere with the infant's immune response to measles vaccination and could cause primary vaccine failure (WHO, 2009a, 2009b). However, if the maternal measles antibodies wane faster than anticipated, the infant is susceptible to measles disease before the age of vaccination (Moss & Griffin, 2012; WHO, 2009b).

Measles vaccine effectiveness when given at 9 months is expected to be about 85%, however this has been shown to vary from as low as 74% to as high as 90% in different countries (Mupere et al. 2006; Uzicanin & Zimmerman, 2011; WHO, 2009a, 2009b). Presence of maternal measles antibodies, malnutrition, cold chain inadequacies, and immature immune system at time of vaccination are some of the factors that contribute to low vaccine effectiveness (WHO, 2009a, 2009b). To enhance vaccine effectiveness to over 95%, in developed countries where measles is not endemic or transmission is very low, measles vaccine is administered at 12 months when there are no more maternal measles antibodies (WHO, 2009a, 2009b). A second dose of measles vaccine is recommended for all children to provide opportunity for an adequate immune response for those that may not have developed protective level of measles antibodies after the first dose. The second dose should be administered through routine

immunization services or supplementary measles campaigns (WHO, 2009a, 2009b). The

second dose should be administered at least one month after the first dose (WHO, 2009a).

In countries where measles is endemic, the second dose should be administered at 15 to

18 months in the routine immunization schedule (WHO, 2009a). However, where

supplementary immunization activities are the delivery channels for the second dose,

children should be targeted for second dose before 5 years (WHO, 2009a).

When an infant is vaccinated with measles, the immune response involves

protective antibody (initially IgM and later IgG) development and development of

measles specific B memory cells (Aurpibul, Puthanakit, Siriaksorn, Sirisanthana, &

Sirisanthana, 2006; Pensieroso et al., 2009; Siegrist, n.d.; WHO, 2009a, 2009b). A person

is considered to have mounted protective levels of measles IgG antibodies if the antibody

titers are equal to or more than 120mIU/ml (Bellini &Rota, 2011; WHO, 2009a, 2009b).

The standard cut off at 120mIU/ml for protective measles antibodies highlighted by

Bellini and Rota (2011), WHO (2009a), and WHO (2009b) was adopted from the work

by Chen et al. (1990) where measles antibody levels of school children were assessed

prior to exposure to a measles outbreak. During the outbreak only children with measles

antibodies less than 120mIU/ml had the clinical presentation of measles disease. Based

on the Chen et al. (1990) study, which involved observation of the effect of natural

exposure to wild measles virus on children with different levels of measles antibodies,

120mIU/ml was recommended as a standard cut off for protective measles antibodies.

Whereas the IgM antibodies are transient and last for about four to eight weeks, the IgG

antibodies last for about three decades, then start declining and may eventually become

undetectable (Moss & Griffin, 2012; WHO, 2009a, 2009b). However, the measles

specific memory cells are maintained and they mount an immunologic memory response,

immediately when a person is infected with measles virus (WHO, 2009a, 2009b).

When a vaccinated person is exposed to wild measles virus, the IgG antibodies if

present protect the person from developing measles illness by neutralizing the virus. In

addition, the measles specific memory cells are stimulated to produce antibodies that are

more protective within one week. Even in the absence of protective levels of measles

antibodies, measles specific memory cells can produce protective antibodies when a

person is exposed to wild measles virus and these antibodies can protect the person from

developing measles illness (Pensieroso et al., 2009; Siegrist, n.d.; WHO, 2009a, 2009b).

The T and B cells start to develop in the body in the first year of life and the immunity of

a person increases as these develop and increase in numbers (Pensieroso et al., 2009).

When a person who has never been vaccinated is infected with measles virus develops

the disease, and survives it, the person develops measles immunity from natural infection.

The immunity development process is similar to that following vaccination; initially IgM

is produced and later IgG. IgM wanes from the blood after about 4 weeks, leaving IgG

antibodies. However, the level of antibodies developed after natural infection is generally

higher than that developed after vaccination (WHO, 2009a, 2009b). Measles IgG

antibodies developed after vaccination last for 26-33 years but those developed following

natural infection stay for a lifetime (WHO, 2009a, 2009b).

Laboratory confirmation of acute measles infection is by either measles virus

isolation from respiratory secretions, nasopharyngeal swabs, blood, or urine specimens

collected within one week of onset of symptoms. Measles can also be confirmed by detection of measles RNA by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) within one week of onset of symptoms. Measles infection could also be confirmed by detection of measles IgM antibodies from blood or oral fluid specimens within four weeks after onset of symptoms, or detection of a 4-fold increase in measles IgG antibodies between acute and convalescent sera (Moss & Griffin, 2012). Since IgM antibodies are also developed after vaccination, history of vaccination in the previous four weeks has to be ruled out when confirming acute measles infection using IgM detection (WHO, 2009b). There are commercially available enzyme linked immunoassay (ELISA) kits for detection of measles IgM and IgG antibodies (WHO, 2009b). In addition, measles IgG antibodies can be quantified by using either a quantitative ELISA or Plaque Reduction Neutralization Test (PRNT) (WHO, 2009b). Cell cultures are used for virus isolation (Moss & Griffin, 2012).

HIV and AIDS

HIV infection is acquired through unprotected sex, vertical transmission from infected mother to child, use of sharp objects with infected blood on them including injection for drug use, and transfusion with infected blood among others (CDC, 2014). In children, the most common source of infection is mother to child transmission (Torpey et al., 2012). An infected mother transfers maternal HIV antibodies to the newborn at birth and these persist in the infant up to 15 to 18 months, irrespective of whether the child is HIV-infected or not (Torpey et al. , 2012; WHO, 2007). The HIV-infected children, in addition have the HIV antigens circulating in their blood (WHO, 2007). Using antibody

detection assays for diagnosis of HIV in children less than 15 to18 months is thus not recommended. An HIV antibody test in such children would detect mainly the maternal antibodies (WHO, 2007). Diagnosis of HIV infection in children less than 15 to 18months (early infant diagnosis) uses antigen detection PCR (Torpey et al., 2012; WHO, 2007).

With HIV infection, the immunity level of an infected individual not on HAART deteriorates through depletion of T cells particularly the CD4+ cells. In addition, there is a decline in number of B cells and abnormalities in their functions that result in hypergammaglobulinaemia, impaired response to vaccination, and loss of antibodies developed after vaccination with different vaccines (Pensieroso et al., 2009). HIV-infected children not on HAART are prone to different types of opportunistic infections. In the pre HAART era, about 50% and 62% died by 2nd and 5th year respectively (Brahmbhatt et al., 2006; Obimbo et al., 2004). Treatment with HAART results in HIV viral control and recovery of the immune system, with increase in CD4+ T lymphocytes and an increase in the B cells. However, immune system recovery following HAART involves production of naïve T cells and probably not an increase in memory cells (Sutcliffe & Moss, 2010). It is uncertain whether the remaining B memory cells maintain or regain their functionality in people on HAART (Pensieroso et al., 2009). There is indication from some studies that HAART started in first year of life could preserve the T and B cell development and functionality. This would ensure that there is minimal or no interference with response to vaccination, and no disruption of the B memory cells function (Pensieroso et al., 2009; Sutcliffe & Moss, 2010).

Measles Antibodies in HIV-infected Children

The effect of HIV infection on measles vaccination response in children has been

studied in different countries, including Uganda, to foster understanding of the possible

risk of measles in HIV-infected children (Aurpibul et al., 2006; Farquhar et al., 2009;

Fowlkes et al., 2011; Kizito et al., 2013; Polonsky et al., 2014; Rainwater-Lovett,

Nkamba, Mubiana-Mbewe, Bolton-Moore, & Moss, 2013; Tejiokem et al., 2007;

Waibale et al., 1999). Studies on the effect of HIV on measles vaccination response

highlight the challenges that HIV infection could pose in measles control and elimination

(Aurpibul et al., 2006; Farquhar et al., 2009; Fowlkes et al., 2011; Kizito et al., 2013;

Moss & Griffin, 2011; Moss & Strebel, 2011; Polonsky et al., 2014; Rainwater-Lovett et

al., 2013; Tejiokem et al., 2007; Waibale et al., 1999). Waibale et al. (1999) conducted a

cross-sectional study to compare the measles vaccination response between HIV-infected

children and HIV-uninfected children in Uganda. In addition, these authors studied the

possible influence of malnutrition on the measles immune response in these children.

This was the first study in Uganda to examine the relationship between HIV infection,

malnutrition, and measles immune response (Waibale et al., 1999).

Contrary to the expected vaccine effectiveness of 85% when measles vaccine is

administered at 9 months (Uzicanin & Zimmerman, 2011), as is done in Uganda, Waibale

et al. (1999) found that 60% of the children enrolled in the study had protective levels of

measles antibodies. Forty percent of the children had low and unprotective levels of

measles antibodies and were considered susceptible to measles. HIV infection and

malnutrition might have contributed to the low proportion of children with protective

measles antibodies since 27% of the children with unprotective antibody levels were HIV-infected, 44% were stunted, and 26% were wasted. Additionally, Waibale et al. (1999) found a significantly higher proportion of HIV-infected children with unprotective antibody level (52%) than HIV-uninfected children (37%). Furthermore, the authors showed significantly higher proportions of stunted and wasted children among HIV-infected children (72% and 40% respectively) than among HIV-uninfected children (28% and 19% respectively). Since stunting, indicative of chronic malnutrition, was a significant predictor of low and unprotective level of measles antibodies, the authors concluded that HIV could be contributing to low measles antibodies through causing chronic malnutrition among the HIV-infected children. Waibale et al (1999) recommended nutritional programs for HIV-infected children and early measles vaccination (by 9 months) of HIV-infected children before T cell function deteriorates due to HIV infection and malnutrition.

The Waibale et al. (1999) study was conducted in the pre-HAART era in Uganda and none of the study participants was on HAART. In the absence of HAART, the contribution of HIV infection to causation of malnutrition in HIV-infected children, and to loss of measles antibodies could not be controlled, thus the findings of Waibale et al. (1999) were significant for measles control in Uganda. The Waibale et al. (1999) findings implied that HIV-infected children were more prone to malnutrition, and the malnutrition affected their response to measles vaccine making them susceptible to measles. From their study results, Waibale et al. (1999) imply that HIV could be a distal determinant and malnutrition a proximate determinant of low unprotective measles antibody, different

from the proximate determinant framework used in this study where both HIV and malnutrition are considered proximate determinants (Boerma & Weir, 2005). However, more studies are needed to understand better the relationship between malnutrition and measles antibodies as well as measles illness in HIV-infected children.

Waibale et al. (1999) used a cross-sectional study design and thus obtained a one-time estimate of the prevalence of measles antibodies. It could not be ascertained from their study whether the low measles antibodies were a result of primary or secondary vaccine failure. In addition, with a cross-sectional study design, the researchers could not study the level of immunity (CD4+ count) and nutrition status at time of immunization and their influence on the measles antibodies. Furthermore, with the cross-sectional design, follow up of the children to determine whether those with HIV infection had a higher incidence of measles than those without HIV infection could not be conducted. A cohort study where HIV-infected and uninfected children's nutritional and CD4+ status is assessed at baseline, both are vaccinated with measles vaccine and followed up overtime after vaccination would have been better. With prospective periodic measurement of measles IgG antibodies, nutritional status, and CD4+ a cohort study would have been enabled better understanding of the relationship between HIV infection and measles immunity, and the effect of malnutrition on the relationship, which were the main objectives of the Waibale et al. (1999) study. However, it would have been more expensive (Creswell, 2009; Mann, 2003). Regardless of the limitations of the cross-sectional design, the Waibale et al. (1999) study findings showed that malnutrition is a major factor contributing to inadequate response to measles vaccination in HIV-infected

children. The protective level of measles antibodies used as a cut off in the Waibale et al. (1999) study (150mIU/ml) was slightly higher than the standard cut off for protective measles antibody level and hence could have resulted in a higher proportion of children with unprotective levels of measles antibodies. Nevertheless, the age group of the children studied was one when children were expected to have very high measles antibodies both from vaccination and from exposure to infection (Tejiokem et al., 2007). Thus using 150mIU/ml as the cut off for protective measles antibodies should not have given more than 15% of HIV-infected and uninfected children with unprotective levels of measles antibodies (Uzicanin & Zimmerman, 2011). Therefore, the finding of about 52% of HIV-infected children with un-protective antibodies (Waibale et al., 1999) was of high concern.

Tejiokem et al. (2007) also conducted a cross-sectional study, in four pediatric care centers (three in Yaounde, Cameroon, and one in Bangui, Central African Republic (CAR)), involving children 18 – 36 months old (mean age was 24.6). These children were born to HIV-infected mothers and had received all EPI vaccines by one year, as documented on their immunization cards. Tejiokem et al. (2007) found that only 20% of HIV-infected children had protective measles IgG. These results are much lower than the 48% found by Waibale et al. (1999) in a similar age group and yet 60% of the children in the study by Tejiokem et al. (2007) were on HAART while none in the study by Waibale et al. (1999) was on HAART. The results obtained by Tejiokem et al. (2007) indicate that even when on HAART, HIV-infected children lose protective measles antibodies. Why there is a 28% difference between the proportion of children with protective

measles antibodies in the Waibale et al. (1999) study in Uganda and the Tejiokem et al. (2007) study in Cameroon and CAR (48% and 20% respectively) remains unclear. However, the results indicate that factors affecting vaccine response in HIV-infected children may differ in the two settings. Aurpibul et al. (2006) conducted a study in older children in Thailand and found that of the 93 HIV-infected children in their study only 41.7% had protective level of measles antibodies (more than 320mIU/ml). Children in the Aurpibul et al. (2006) had an average age of 9.7 years compared to children in the Waibale et al. (1999) study who were between 1.4 and 3.4 years old (16.8 to 41.3 months) and those in the study by Tejiokem et al. (2007) who were 1.5 to 3 years old. The proportion of HIV-infected children with protective measles antibodies in the Aurpibul et al. (2006) study was even lower (36.5%) when only the participants with validated measles vaccination records were considered. Interestingly only 42.6% of the children with 1 dose and 22.2% of the children with 2 doses documented had protective measles antibodies in the Aurpibul study. These results contrast with the expected higher prevalence of protective measles antibodies in children who have received 2 measles vaccine doses at appropriate ages (Aurpibul et al., 2006; Uzicanin & Zimmerman, 2011).

About 60% of the HIV-infected children in the Tejiokem et al. (2007) study were on HAART, and 62% of those on HAART had been on the treatment for more than 6 months. Yet only 20% had protective measles antibodies indicating that HAART may not prevent loss of measles antibodies in HIV-infected children. Aurpibul et al. (2006) found that more than half of the HIV-infected children on HAART, with reconstitution of the immune system, did not have protective measles antibodies levels, and were potentially

susceptible to measles infection. Furthermore, Aurpibul et al. (2006) found that the age at HAART initiation was not a significant predictor of level of measles antibodies. On the contrary, in a later study, Pensieroso et al. (2009) found that starting children on HAART before vaccination with measles and before deterioration of the B cells functions could prevent loss of protective measles antibodies. However, the results of these two studies are difficult to compare because children in the Aurpibul et al. (2006) and the Pensieroso et al. (2009) studies started HAART at different ages. While in the Aurpibul et al. (2006) study, the average age at HAART initiation was 7 years, in the Pensieroso et al. (2009) study HAART was started at a mean age of 6.8 months. Since the effect of age at initiation of HAART on measles antibodies is not well known, more studies are needed to add more data and broaden the understanding on the potential effect of timing of antiretroviral treatment on measles immunity following vaccination. The proportion of HIV exposed uninfected children with protective measles antibodies found by Tejiokem et al. (2007) of 56% was similar to the proportion of HIV-uninfected children with protective measles antibodies in the Waibale et al. (1999) study (63%). These results indicate a much higher than expected vaccine failure in HIV-uninfected children (Uzicanin & Zimmerman, 2011) and may suggest the presence of additional factors not related to HIV infection that may cause vaccine failure in the study communities.

In the Tejiokem et al. (2007) study, HIV-infected children with a CD4+ count below 25% had significantly lower levels of measles antibodies compared to those with CD4+ count above 25% implying that low CD4+ count could be a predictor of low level of measles antibodies and possible higher susceptibility to measles. In contrast, Aurpibul

et al. (2006) found that CD4+ count, was not significantly associated with, and was not a predictor of the level of measles antibodies. The average CD4+ count among the participants without measles antibodies was 24.8% while that among the participants with measles antibodies was 24.5% (Aurpibul et al., 2006). Similarly, not being on HAART or having been on HAART for less than 6months, and having a viral load above 10,000 copies per ml. were associated with low levels of measles antibodies in the Tejiokem et al. (2007) study. These findings may indicate that measles antibodies did not develop or waned in individuals with a high HIV viral load. Consistent with the finding by Tejiokem et al. (2007), Abzug et al. (2010) found that very low HIV viral load (less than 400 copies per ml) was a predictor of protective measles antibodies in HIV-infected children. This finding indicates that it may be more of the HIV virus load than the CD4+ count per se that is associated with declining measles antibodies (Abzug et al., 2010). However, this contradicts the finding by Tejiokem et al. (2007) that suggests low CD4+ could be a predictor of low measles antibodies in HIV-infected children; thus inconsistent information on low CD4+ as a predictor of loss of measles antibodies and risk of measles. Similarly, there is inconsistent information on the role of malnutrition in the loss of measles antibodies.

Tejiokem et al. (2007) found no significant association between nutrition status and measles antibody level, different from what was shown by Waibale et al. (1999). The main measure of nutrition status in the Tejiokem et al. (2007) study was Mid Upper Arm Circumference (MUAC) for age, which is one of the measures for acute malnutrition (wasting) in children less than 5 years of age (Shetty, n.d). When MUAC was less than 2

Z score the child was considered to be malnourished. However, since severely sick and severely malnourished children were excluded from the Tejiokem et al. (2007) study, the finding may not be conclusive about the relationship between nutrition status and measles antibody response (Tejiokem et al., 2007). Regarding vaccination, interestingly, there was no significant difference between children with one measles dose and those with 2 measles doses and between the time since last vaccination and measles antibody levels (Tejiokem et al., 2007), contradicting findings by Fowlkes et al. (2011). In the Fowlkes et al. (2011) study measles antibodies declined with age and time since last vaccination, especially in HIV-infected children.

Although Tejiokem et al. (2007) observed a significant reduction in measles antibodies in HIV-infected children, the authors could not predict whether the HIV-infected children with low or no measles antibodies were susceptible to measles. In HIV-uninfected children, not exposed to HIV during pregnancy, the immune response developed after vaccination is long lasting, such that even when measles antibodies reduce to below protective level, the B cell memory response is protective against measles infection (Pensieroso et al., 2009; Siegrist, n.d). In HIV-infected children, the reduction in CD4+ and B cells and their deficiencies might interfere with the B cell memory response making children with low or no detectable measles antibodies susceptible to measles (Pensieroso et al., 2009; Tejiokem et al., 2007). The risk of measles in children with HIV and unprotective measles antibodies has not been studied to ascertain whether the B cell memory is completely lost and is not protective against measles. In children on HAART, there is improvement of the CD4+ count and the

general immune system (Sutcliffe & Moss, 2010). However, it is not clear whether the improvement in the immune system maintains the B cell memory response when treatment is started early, or whether it prevents or modifies the decline of measles antibodies. If it does, putting HIV-infected children on HAART early could reduce susceptibility of these children to measles. If it does not, HIV-infected children surviving longer due to HAART could be highly susceptible to measles due to secondary vaccine failure. Sutcliffe and Moss (2010) suggested that HAART does not restore immunity to measles if it had already waned before treatment initiation and neither does it restore immunologic memory. Tejiokem et al. (2007) recommend more studies, to assess further the effect of early initiation of HAART on response to EPI vaccines.

Consistent with the findings by Kizito et al. (2013) and Waibale et al. (1999), Tejiokem et al. (2007) found low levels of antibodies in HIV-infected and uninfected children, sampled from four pediatric centers in CAR and Cameroon in Central Africa respectively. Yet the children's antibodies were expected to have peaked due to vaccination and exposure to measles infection. Kizito et al. (2013) findings were based on a sample of HIV-infected and uninfected children born to mothers who lived in Entebbe in Wakiso district of Uganda, while the findings by Waibale et al. (1999) were based on a sample of HIV-infected and uninfected children attending the general and HIV clinics at Mulago Hospital in Kampala, Uganda. These findings could indicate that several infections including malaria and HIV, among others, and levels of moderate malnutrition highly prevalent in the developing countries in Sub-Saharan Africa could be affecting the development or maintenance of measles antibodies in the children (Kizito et

al., 2013). The effect of the above infections on development or maintenance of measles antibodies could be varying in different countries based on the disease burden (Kizito et al., 2013).This finding could have implications for measles transmission in Sub-Saharan Africa because the high HIV prevalence coupled with high prevalence of other infections and malnutrition, could result in high incidence of measles in both the HIV-infected and HIV-uninfected children hampering measles control efforts. Tejiokem et al. (2007) used a cross-sectional study design and compared HIV-infected with HIV exposed uninfected but there was no comparison with HIV unexposed uninfected children in their study. HIV exposed uninfected children may also be having challenges with response to vaccinations (Abramczuk et al., 2011). Hence, inclusion of another reference group, the HIV unexposed uninfected as in the study by Fowlkes et al. (2011), would have provided additional and valuable information.

Kizito et al. (2013) conducted a secondary analysis of specimens and data collected in a randomized controlled trial in Entebbe, Uganda, that recruited women in 2nd or 3rd trimester, to assess the effect of antihelminthes treatment during pregnancy on the immune response of infants to vaccination. This secondary analysis was based on 711 pairs of pregnant women and their infants from whom stored blood specimens collected at enrollment, at delivery (from mother and cord blood), and from the vaccinated infants at 1 year were available. Kizito et al. (2013) found that 75% of the infants had protective levels of antibodies at 1 year; only 3 months after vaccination. However, the authors used the protective measles antibody cut off level of 200mIU/ml and not the standard cut off level of 120mIU/ml. This higher cut-off could have resulted in underestimation of the

proportion of children with protective measles antibodies 3 months after vaccination,

indicating a higher vaccination failure rate than the expected 15% when the vaccine is

given at 9 months (Uzicanin & Zimmerman, 2011). It is not clear whether the vaccine

failure was primary or secondary but given the months after vaccination, this is likely to

have been primary vaccine failure. Furthermore, maternal measles antibody level among

the infants was not assessed at vaccination yet the mothers had very high levels of

measles antibodies (average of more than 4,000 mIU per ml). Kizito et al. (2013) found

that malaria infection in the mothers during pregnancy, infant malaria parasitaemia, HIV

infection in the infants acquired through mother to child transmission, and infant wasting

were associated with unprotective measles antibodies among the infant. The study

highlights the contribution of HIV infection and other infections like malaria, and

malnutrition (wasting) to measles vaccine failure and their possible contribution to

measles susceptibility and measles outbreaks in measles endemic areas. However, Kizito

et al. (2013) did not show whether the measles vaccine failure in HIV-infected children

actually predisposed them to higher risk of measles in outbreak situations in Uganda than

that of HIV-uninfected children.

Whereas the findings pertaining to HIV infection are consistent with other studies

that have shown declining measles antibodies in HIV-infected children, Kizito et al.

(2013) findings related to infant wasting contradict the finding by Tejiokem et al. (2007)

that showed no effect of malnutrition (wasting) on immune response to measles

vaccination. The findings by Kizito et al. (2013) indicating that wasting (indicative of

acute malnutrition) was associated with low unprotective measles antibodies and the

results by Waibale et al. (1999) indicating stunting (indicative of chronic malnutrition) as a significant factor associated with unprotective measles antibodies create an impression that measles vaccine failure associated with different forms of malnutrition might be a problem in Uganda and not in other countries. Besides, what is diagnosed as malnutrition in the studies by Kizito et al. (2013) and Waibale et al. (1999) may be an effect of several infections that affect children in the first 5 years of life that keep challenging the immune system and also affect measles antibody levels (Alilio et al., 2004; WHO, 2009). However, none of the studies reviewed analyzed the effect of other infections suffered by the children other than HIV on the level of measles antibodies.

Fowlkes et al. (2011) conducted a clinical trial aimed at comparing the response to one and two doses of measles vaccine among HIV-infected and HIV-uninfected children at Ndirande Health Center near Blantyre, Malawi. In the Fowlkes' study, children born to HIV-infected mothers were given measles vaccine at 6 and 9 months; while other children including HIV-exposed uninfected and HIV-unexposed uninfected children, were randomized to receive measles vaccine at either 6 and 9 months, or as a single dose at 9 months. The sample included 250 HIV-uninfected children vaccinated at 6 and 9 months, 250 HIV-uninfected children vaccinated at 9 months only, and 72 HIV-infected children vaccinated at 6 and 9 months (Fowlkes et al., 2011).

The results by Fowlkes et al. (2011) showed a decline in the proportion of children seropositive for measles antibodies as the children grew; although this decline was more marked in the HIV-infected children than in HIV-uninfected groups. By 20 months of follow up, 40% of HIV-infected children had protective antibodies against

measles, and by 24 months (2 years) none of the HIV-infected children followed up had

protective measles antibody levels (Fowlkes et al., 2011). Furthermore, 13% of all the

children that had measles antibodies at 12 months did not have detectable antibodies by

20 months. HIV-infected children were found 2.3 times more likely to have undetectable

measles antibodies compared to the HIV-uninfected children vaccinated at 6 and 9

months (Fowlkes et al., 2011). The study design was very appropriate for the Fowlkes'

study to enable follow up and analysis of the measles antibodies over several months

after vaccination. The dropout rate in the study was quite significant although the authors

indicate that there were no differences in demographic characteristics between children

who stayed in the study and those who were lost to follow up and hence did not pose

validity threats to the results. With the high dropout rate, the numbers of HIV-infected

children were particularly very small by 20 and 24 months (23 and 3 respectively); for

59% of the 58 HIV-infected children death was the reason for loss to follow up.

Contrary to what the authors indicate, the small number of children followed up at

20 and 24 weeks poses external and internal validity threats for this study. At the time the

Fowlers' study was conducted, HAART was not yet available in the public health sector

in Malawi and HIV infection was associated with low survival of the infected children by

2 to 5 years (Brahmbhatt et al., 2006; Obimbo et al., 2004). Nevertheless Fowlkes et al.

(2011) indicated that about 60% of HIV-infected children were lacking protective

measles antibodies by 20 months, and that all HIV-infected children alive by 24 months

had no protective antibodies and were thus possibly susceptible to measles. The study

however, did not show whether the children without protective antibodies had a higher

risk or incidence of measles illness. Whereas the study design could have allowed the researchers to compare measles incidence in HIV-infected and HIV-uninfected children, the study was conducted at a time when there was very low measles transmission in the country (Fowlkes et al., 2011). Hence differences in incidence of measles could not be ascertained. Due to the small numbers of HIV-infected children followed up at 20 and 24 months, the results of the study may not be conclusive regarding the proportion of vaccinated HIV-infected children with protective levels of measles antibodies at this age. The results may over or underestimate the proportion of HIV-infected children without protective antibodies and thus possibly susceptible to measles by this age.

In the Fowlkes et al. (2011) study, there were 10 children who seroconverted to HIV at more than 12 months of age. These children were vaccinated before the HIV infection. The response to measles vaccine among these children was similar to that among the HIV-uninfected children who received measles vaccine at 6 and 9 months. This finding may indicate that HIV infection after vaccination may not result in secondary vaccine failure as experienced when HIV-infected children are vaccinated. Based on results by Fowlkes et al. (2011) and Pensieroso et al. (2009), it is possible that the initiation of HAART in HIV-infected children at an early age before immunity deteriorates and before measles vaccination, may give similar results as with children who seroconverted after 12 months. Early initiation of HAART (before the 1st dose of measles vaccine at 6 months) could thus be associated with reduced risk and odds of measles in HIV-infected children (Pensieroso et al., 2009). The recommendation by WHO that HIV-infected children should be started on HAART soon after diagnosis,

regardless of the CD4+ count (WHO, 2013), adopted by Uganda (Uganda Ministry of Health, 2013) is in line with findings by Fowlkes et al. (2011) and Pensieroso et al. (2009). However, whether early initiation of HAART will result in maintenance of measles antibodies in HIV-infected Ugandan children and similar levels of measles incidence in HIV-infected and HIV-uninfected remains unknown now.

Fowlkes et al. (2011) did not assess the CD4+ count and viral load of HIV-infected children and thus could not ascertain if severe immune suppression and high viral load contributed to the declining and or loss of detectable measles antibodies as had been indicated by Tejiokem et al. (2007). Whereas the nutrition status of the study participants at birth and at 18 months was recorded, the authors did not assess the relationship of nutrition status and the response to measles vaccination (Fowlkes et al., 2011). As a result, the Fowlers' study did not generate information to confirm or contradict other studies (Kizito et al., 2013; Waibale et al., 1999) that indicated that acute and chronic malnutrition were associated with inadequate response to measles vaccination or the study by Tejiokem et al. (2007) that showed that acute malnutrition was not associated with inadequate response to measles vaccine. Given the lack of consistency of the findings on the relationship between malnutrition and secondary vaccine failure in HIV-infected children, more studies are needed to clarify the relationship if any.

The findings from Fowlkes et al. (2011) showed an initial good rate of sero-conversion by 12 months followed by a decline in the measles antibodies, indicating that HIV infection caused secondary measles vaccine failure in a cohort of HIV-infected

children not on HAART. The effect of HIV on measles vaccine response was hence clear in the Fowlkes' study, unlike in the cross-sectional studies by Kizito et al. (2013), Tejiokem et al. (2007), and Waibale et al. (1999), where it was unclear whether vaccination failure in the HIV-infected children was primary or secondary. Since the study setting was in an area with very low measles transmission at the time (Fowlkes et al., 2011), the measles antibodies the children had were mainly a response to measles vaccination. As the children that had reduced or loss of the measles antibodies were not exposed to wild measles virus, their susceptibility to measles infection or protection from B cell memory response could not be ascertained. More studies to elucidate the effect of the reduction and eventual loss of measles antibodies in HIV-infected children on HAART on measles incidence in this group of children, especially in endemic communities and outbreaks, are needed.

Aurpibul et al. (2006) conducted a cross sectional study involving 96 HIV-infected children in Chiang Mai University Hospital, Chiang Mai, Thailand, in 2005. Aurpibul et al. (2006) collected data on several parameters, including previous illnesses suffered by the children and some retrospective data from medical records and interviews with parents, guardians, or caretakers. Aurpibul et al. (2006) found that 41.7% of the 93 HIV-infected children had protective level of measles antibodies (more than 320mIU/ml). Additionally, Aurpibul et al. (2006) found no significant difference between those with protective measles antibodies and those without in terms of gender, CD4+ count prior to HAART, duration of immune suppression, age at which HAART was initiated, period between last measles vaccination and collection of specimens for measles IgG testing,

and current CD4+ percentage (Aurpibul et al., 2006). This finding could imply that CD4+ percentage, i.e. level of immune suppression, duration of the immune suppression, and age at which HAART is initiated are not significant predictors of protective measles antibodies and measles susceptibility in HIV-infected children. The findings are quite surprising as it would be expected that the more immune suppressed would have lower antibody levels. The above findings by Aurpibul et al. (2006) contradict that by Pensieroso et al. (2009) that indicated that early initiation of HAART could prevent decline in measles antibodies. However, given that the participants with average age of 9.7 years and had been on HAART for about 24 months, meaning that HAART was started on average around 7.7 years, it is likely that the HIV infection had already caused the loss of measles antibodies by the time of initiation of HAART and that immune reconstitution could not reverse the antibody loss. In addition, the finding that the time elapsed from last vaccination did not significantly affect antibody levels contradicts the findings by Fowlkes et al. (2011) that indicated antibody levels decreased with time since vaccination. It was also interesting to note that by 9 years (average age of the participants in this study), about 40% of the participants in the study in Thailand had protective antibodies following infant vaccination while in studies in Sub-Saharan Africa $0-20\%$ had protective measles antibodies by $2-3$ years (Fowlkes et al., 2011; Tejiokem et al., 2007).

Due to the cross-sectional nature of the study by Aurpibul et al. (2006), the authors could not ascertain whether the lack of protective antibodies was due to primary or secondary vaccination failure. In addition, Aurpibul et al. (2006) did not measure the

percent of CD4+ at the time of vaccination and could not determine whether some of the children were immune compromised. Having a severe immune suppression at time of vaccination would likely result in primary vaccine failure and therefore measles vaccination is not recommended by WHO in severely immune compromised children (Aurpibul et al., 2010). The lack of a comparison group in the Aurpibul et al. (2006) study is also a limitation that prevents comparison of the prevalence of measles protective antibodies among the HIV-infected and the HIV-uninfected children in Thailand. Comparison between the HIV-infected and HIV-uninfected children could have facilitated more understanding of the contribution of HIV infection to lack of protective levels of measles antibodies and possibly measles susceptibility in Thailand. Aurpibul et al. (2006) considered mainly antibody response and not the B cell memory response. In addition Aurpibul et al. (2006) did not assess for history of measles in the HIV-infected children in spite of the study children being at an age (9 years) where they were more likely to have been exposed to wild measles virus. Thus Aurpibul et al. (2006) did not ascertain whether the participants without protective levels of measles antibodies were susceptible to and at high risk of measles.

What is consistent in all the studies reviewed above is that there is waning of measles antibodies in HIV-infected children and this makes them potentially susceptible to measles (Aurpibul et al., 2006; Fowlkes et al., 2011; Kizito et al., 2013; Sutcliffe &Moss, 2010; Tejiokem et al., 2007; Waibale et al., 1999). These children could accumulate into a pool of susceptible children in communities or countries with high HIV prevalence to maintain measles transmission, produce measles outbreaks, and hamper

attainment of measles control goals (Moss & Strebel, 2011; Sutcliffe & Moss, 2010). Helfand, Perry, and Strebel (2007) argue that such susceptible children should be protected by herd immunity in their communities through attainment of high vaccination coverage (equal to or more than 95%) (Moss & Strebel, 2011). Given that, it is quite difficult, especially in Sub-Saharan countries, to achieve and maintain vaccination coverage equal to or more than 95% (WHO Regional Office for Africa, 2012), this suggestion is not feasible yet in Sub-Saharan Africa.

Studies on measles vaccine response reviewed used different cut offs to assess protective level of measles antibodies, making comparison of their results difficult (Aurpibul et al., 2006; Fowlkes et al., 2011; Kizito et al., 2013; Tejiokem et al., 2007; Waibale et al., 1999). Future studies on measles vaccine response in HIV-infected children on or not on HAART should use 120mIU/ml, the standard cut off for protective measles antibodies, to enable comparability of results. In addition, the sample of HIV-infected children in previous studies has been small, making generalization of the findings a challenge (Fowlkes et al., 2011; Tejiokem et al., 2007; Aurpibul et al., 2006).The regional differences in prevalence of protective measles antibodies also make generalization of results beyond regions inappropriate (Aurpibul et al., 2006; Fowlkes et al., 2011; Tejiokem et al., 2007; Waibale et al., 1999).

Role of HAART in Rebuilding Immunity Levels

The introduction of HAART has resulted in survival of HIV-infected children to adolescents and young adults (Banerjee, Pensi, Banerjee, & Grover, 2010). The immune reconstitution that occurs in children on HAART results in increase in CD4+ count and B

cells however, the effect of HAART on maintenance or regeneration of B memory cells remains unclear (Banerjee et al., 2010; Pensieroso et al., 2009). Pensieroso et al. (2009) conducted a cross-sectional study that involved evaluation of 70 HIV-infected children attending a hospital in Rome, Italy. Pensieroso et al. (2009) found that the levels of B memory cells among the early treated HIV-infected children (started on HAART at an average age of 6.8 months) was comparable with that among healthy controls. They also found that there was a significant difference in the levels of B memory cells of early treated and late treated children (started HAART at an average age of 7.4 years), and between late treated children and healthy controls. The finding by Pensieroso et al. (2009) implies that early initiation of HAART could result in maintenance of the B memory cells in HIV-infected children at a level similar to healthy HIV-uninfected children, preventing the decline in B memory cells experienced with HIV infection. Pensieroso et al. (2009) also found no difference between the measles specific Eli Spots, a measure of measles specific memory cells, of the early treated and the healthy control groups. However, there was a significant difference in the EliSpots of the early treated and late treated, with the latter showing significantly lower Eli Spots. These results by Pensieroso et al. (2009) indicate that early initiation of HAART could maintain measles specific memory cells among the HIV-infected children at the same rate as healthy children.

Regarding the measles antibody levels, Pensieroso et al. (2009) found that 100% of healthy controls and 82% of early treated group had protective measles antibody levels, while 39%, 40%, and 33% of the late control, late failure, and treatment naïve

groups respectively had protective levels of antibodies. These findings imply that starting HAART within the first year of life could result in maintenance of protective levels of measles antibodies in children on HAART and hence prevent secondary vaccine failure seen in HIV-infected children (Aurpibul et al., 2006; Fowlkes et al., 2011; Kizito et al., 2013; Pensieroso et al., 2009; Tejiokem et al., 2007; Waibale et al., 1999). The level of measles antibody in the Pensieroso et al. (2009) study was not influenced by the time since last measles vaccination for the healthy controls and early treated group; some of these still had protective levels of antibodies by 7 to 10 years. However, for the late treated and treatment naïve groups, several of the children had below protective level of measles antibodies by one year after vaccination, similar to findings by Kizito et al. (2013). Pensieroso et al. (2009) relate the maintenance of protective antibodies in early treated patients to maintenance of the B cells functions. Besides there was a positive correlation between antibody titers and number of measles specific Eli Spots, an indication of correlation between the measles antibody titers and the presence of B memory cells (Pensieroso et al., 2009).

Pensieroso et al. (2009) highlighted the effect of early initiation of HAART on the immune system of HIV-infected children. In their study, early initiation of HAART, in the first year of life, in HIV-infected children prevented secondary measles vaccine failure by preventing waning of antibodies and maintaining the measles specific memory cells. Early initiation of HAART could thus enhance attainment of measles control in countries like Uganda with high HIV prevalence. However, Pensieroso et al. (2009) did not examine the effect of early and late initiation of HAART on actual incidence of

measles. The sample size in the Pensieroso et al. (2009) study was very small. Out of 70

HIV-infected children, only 13 were treated early and there were only 6 treatment naive

(Pensieroso et al., 2009). Thus, it is difficult to generalize the results to all HIV-infected

children on or not yet on HAART. The nutrition status of children in the Pensieroso et al.

(2009) study as well as other infections that could have affected the children between the

vaccination and the time of the study were not taken into consideration. However, they

could play a role in the loss of antibodies in some of the children, particularly the HIV-

infected children.

Le Roux, LeRoux, Nuttall, and Eley (2012) reported on a retrospective review of

a measles outbreak in South Africa in 2009 – 2010 as seen in Red Cross War Memorial

Hospital in Cape Town. A total of 1,861 children were seen at the hospital with measles,

of which 552 were admitted with severe measles disease and 18 of the admitted children

died at the hospital. The median age for the admitted patients was 7.36months. Le Roux

et al. (2012) found that 14% of the admitted were HIV-infected children, 51% of whom

had been on HAART for more than 3 months. HIV-infected children had seven times

higher odds of death due to measles than HIV-uninfected children. The results of Le

Roux et al. (2012) could imply that early initiation of HAART may not have been

protective against measles vaccine failure, contradicting findings by Pensieroso et al.

(2009). However, in the Le Roux et al. (2012) study, only 47% of the children had

received a dose of measles vaccine at 6 months and most had not yet received a second

dose given at 9 months due to young age. The finding by Le Roux et al. (2012) that HIV-

infected children less than 9 months were at risk of measles probably due to low and

rapidly waning maternal antibodies was consistent with the finding by the deMorales-Pinto (2011) review. However, among the 47% that had been vaccinated at 6 months, waning antibodies may not have been the reason for susceptibility; it is unclear whether measles antibody avidity could have played a role in the susceptibility of these children to measles. Measles illness in vaccinated HIV-infected children, started on HAART before vaccination, as seen in the Le Roux et al. (2012) study contrasts with Pensieroso et al. (2009) finding that indicated that starting HAART in the first year of life prevents vaccine failure. More studies to examine the effect of age at initiation of HAART on measles antibodies and measles incidence in the HIV-infected children are needed.

Risk of Measles in HIV-infected Children on HAART and Possible Predictors

Several studies have shown a decline in measles antibodies in HIV-infected children on HAART (Aurpibul et al., 2006; Tejiokem et al., 2007). In measles endemic countries or countries that currently experience measles outbreaks, HIV-infected children may be at very high risk for measles (Moss & Strebel, 2011). Sub-Saharan Africa with the highest HIV prevalence has experienced several measles outbreaks in the last 3 years (CDC, 2013) raising questions about the contribution of HIV infection to the outbreaks. While there is increasing access to HAART by HIV-infected children in Sub-Saharan Africa resulting in their increased survival, it is not clear whether due to the secondary vaccine failure noted in these children they are proportionately more affected by measles in measles outbreaks in Sub-Saharan Africa. Whereas this has not been comprehensively studied, Nilsson and Chiodi (2011), based on a systematic review of related literature, found that inadequate response to measles vaccination in HIV-infected children was

related to a reduction in memory cells and inability to mount immunologic memory. Nilsson and Chiodi (2011) suggested that the secondary vaccine failure in HIV-infected children with loss of immunologic memory capacity might be a major contributor to measles outbreaks in countries with high HIV prevalence in Sub-Saharan Africa.

Le Roux et al. (2012) found that inadequate vaccination coverage, age of affected children, and loss of maternal antibodies at an early age were factors that contributed to the high risk of measles for the HIV children in South Africa during the 2009 – 2010 measles outbreak. However, there was no comparison between vaccinated HIV-infected children on HAART, vaccinated HIV-infected children not on HAART, and vaccinated HIV-uninfected children in the same age groups. This was mainly due to some data missing from the medical records that were the source of information in their study (Le Roux, et al., 2012). Hence, the findings of the Le Roux et al. (2012) study are not conclusive about the risk factors and the risk of measles in HIV-infected children on HAART.

Sartorius et al. (2013) studied the risk factors for measles outbreaks and identified high-risk areas for possible measles outbreaks using data from the South African measles outbreak of 2009 to 2011. Sartorius et al. (2013) found that the measles outbreak in South Africa was associated with a proportion of susceptibles in affected districts exceeding 20%, a high population density, and a high prevalence of HIV. This finding is consistent with the suggestion by Nilsson and Chiodi (2011) that measles outbreaks in Sub-Saharan Africa may be linked to high HIV prevalence in the countries affected. However, whereas the model used by Sartorius et al. (2013) considered the longer survival of children on

HAART, it did not consider the risk of measles in HIV-infected children on HAART compared to HIV-uninfected children. Whereas the Sartorius et al. (2013) model highlighted high HIV prevalence as a factor contributing to the outbreak, it is not indicated whether being on HAART reduced or increased the risk of measles in the outbreak. More studies to assess this in outbreak situation are needed.

Following a measles outbreak in Kampala from February to June 2012, Korutaro et al. (2013) conducted a retrospective review of records of HIV patients younger than 14 years attending the pediatric HIV clinic at Baylor College of Medicine Children's Foundation, in Kampala, Uganda. The review focused on records from January to June 2012 with the objective to understand and describe the effect of measles outbreak on HIV-infected children. Korutaro et al. (2013) found that 135 of the children attending the clinic during the 6 month period were diagnosed with measles, 109 (81%) of whom were on HAART. The average CD4+ percentage for the children was 28% indicating that they had already experienced immune reconstitution. Korutaro et al. (2013) found no association between CD4+ percentage, HAART use, or stage of HIV with measles occurrence. However, malnutrition (wasting and underweight) were independently associated with measles occurrence in the HIV-infected children on or not on HAART. Besides, all the four deaths occurred in malnourished children.

Since measles antibodies are not measured routinely at the pediatric HIV clinic at Baylor College of Medicine Children's Foundation, in Kampala, it was not possible to ascertain the level of measles antibodies prior to measles illness. Whereas the authors do not comment about the vaccination status, the pediatric HIV clinic at Baylor College of

Medicine Children's Foundation, in Kampala is one of the best pediatric HIV clinics in

Uganda and the HIV patients attending the clinic receive all the vaccines offered in

routine immunization schedule in Uganda timely (Baylor International Pediatric AIDS

Initiative, 2011). Thus, it is expected that most or all of the children in the study had

received at least 1 dose of measles vaccine at 9 months and or during measles campaigns.

However, the fact that these HIV-infected children on HAART, with immune

reconstitution, developed measles is an indication that they were susceptible to measles.

The findings highlight different forms of malnutrition (wasting and underweight) as

independent factors associated with measles occurrence in HIV-infected children in

Uganda. Although Waibale et al. (1999) found that malnutrition rather than HIV per se

was associated with inadequate response to measles vaccination in HIV-infected children

in Uganda, the significant form of malnutrition contributing to inadequate response to

measles vaccine was stunting. These results indicate that the different forms of

malnutrition (stunting, wasting, and underweight) may be independent factors

contributing to primary and secondary measles vaccine failure. Korutaro et al. (2013) did

not include a comparison group of HIV-uninfected children and thus it was not possible

to ascertain whether the HIV-infected children were proportionately more affected by

measles in the measles outbreak than the HIV-uninfected children.

Several studies also tried to identify possible predictors of low and unprotective

measles antibodies that could thus be predictors of high risk for measles in HIV-infected

children. However, findings have not been consistent because some factors that are found

to be significantly associated with unprotective level of measles antibodies in some

studies are found not associated with unprotective measles antibodies in other studies

(Sutcliffe & Moss, 2010). For example, Pensieroso et al. (2009) found that early initiation

of HAART, in the first year of life, was a predictor of reduced risk of measles in HIV-

infected children as it prevented waning of antibodies and loss of measles specific

memory cells. However Abzug et al. (2010) found that age at initiation of HAART was

not a significant predictor of level of measles antibodies. Abzug et al. (2010) showed

inverse correlation between the HIV viral load and level of measles antibody, with a viral

load less than 400 copies/ml correlating with the highest antibody levels in HIV-infected

children on HAART. Abzug et al. (2010) indicate that it may be the HIV viral

suppression or replication that influences the waning of measles antibodies, and not a

reduction in CD4+ percentage or absolute numbers. Age, sex, and CD4+ percentage prior

to and even while on HAART were all not significant predictors of protective measles

antibodies in the study by Abzug et al. (2010). However, no study examining CD4+

count relationship with measles antibodies and incidence has been done in Uganda where

there is high prevalence of HIV and increasing access of HIV-infected children to

HAART.

Malnutrition has been indicated in several studies as a possible predictor of risk of

measles in HIV-infected children (Kizito et al., 2013; Korutaro et al., 2013; Waibale et

al., 1999). The relationship between HIV infection and malnutrition in regards to the

association with measles occurrence is not clear yet. While Waibale et al. (1999) showed

that chronic malnutrition (stunting) in HIV-infected children affected the response to

measles vaccination and not the HIV infection per se, Kizito et al (2013) found that HIV

infection and acute malnutrition (infant wasting) were both independent predictors of unprotective measles antibodies. Korutaro et al. (2013) found that malnutrition (wasting and underweight) was a predictor of high risk of measles in HIV-infected children. Based on results by Kizito et al. (2013), and Korutaro et al. (2013), malnutrition (wasting and underweight) could thus be a proximate determinant in the relationship between HIV infection and measles. Furthermore, from the Waibale et al. (1999) results malnutrition (stunting) could be a proximate determinant of measles in HIV-infected children. More studies to examine if this is the case are needed.

Revaccination of Children with HIV on HAART

Given that measles antibodies wane in HIV-infected children, especially when HAART is not started in the first year of life (Aurpibul et al., 2006; Pensieroso et al., 2009), there is need to ascertain if revaccination of HIV-infected children on HAART, after immunity has been reconstituted, could result in development and maintenance of protective levels of measles antibodies. Melvin and Mohan (2003) followed up children in routine HAART care in Seattle, Washington, who had been revaccinated with different vaccines, including measles, when their disease specific antibodies were undetectable on routine testing. Melvin and Mohan (2003) found that 83% of the children revaccinated had detectable measles antibody levels by 4 weeks post vaccination. By 12 months, 8/11 (73%) followed up still had detectable measles antibodies, with minimal, nonsignificant difference in the levels of antibodies detected at 4 weeks and 12 months post vaccination. Melvin and Mohan (2003) showed that when children are on HAART, routine assessment of antibodies to vaccine preventable diseases against which they have been

vaccinated is necessary. Furthermore, the authors showed that on revaccination of those with undetectable antibodies, most of them developed detectable antibodies that lasted for at least 12 months (Melvin & Mohan, 2003). It is however, not clear from the Melvin and Mohan (2003) study results whether the detected antibodies post vaccination reached or exceeded protective levels. In addition, the number of participants in this study was small (19) for results to be generalized beyond the study population, there was no comparison group, and the follow up was only for 12 months. By 12 months post revaccination, there was a small decline in proportion of children with detectable antibodies from 83% to 73%. However, the authors do not indicate whether this decline was statistically significant (Melvin & Mohan, 2003). Due to the study follow-up period it is not known whether, there was an additional decline in the antibodies after 12 months and whether antibodies were lost after some time. Thus, more studies with longer follow up and quantification of antibodies detected as done by Abzug et al. (2010) and Aurpibul et al. (2010) are more informative on the response to measles revaccination in HIV-infected children on HAART.

In a follow up study of HIV-infected children on HAART in Thailand by Aurpibul et al. (2010), 51 children with immune recovery (defined as CD4+ percentage of greater than 15% for 3 consecutive months) following different periods on HAART were revaccinated with a dose MMR and followed up for 40 months. The aim of the study was to assess to if protective measles antibodies persist 3 years after revaccination of HIV-infected children on HAART. Aurpibul et al. (2010) found that 85% of the 38 children followed up had protective level of measles antibodies at 40 months post

revaccination, however there was significant reduction in the geometric mean titers over the years of follow up. Two children that had protective measles antibodies 6 months post revaccination were seronegative for measles antibodies at 40 months indicating waning of antibodies even in some children with reconstituted immunity (Aurpibul et al., 2010). However, the number of children whose antibodies waned (2) is so small that this finding is inconclusive; besides the authors do not indicate if this finding is statistically significant. Aurpibul et al. (2010) thus demonstrated persistence of protective measles antibodies in a high proportion (85%) of HIV-infected children with immune recovery following HAART, for at least 3 years following revaccination with MMR. However, the authors did not indicate what proportion of the children had no protective antibodies before revaccination. If some of the children had protective antibodies prior to revaccination, this could result in overestimation of the proportion of children with protective measles antibodies post revaccination.

Aurpibul et al. (2010) also showed that in some individuals, even with immune recovery, the measles antibodies still wane after revaccination. Booster doses may be needed for such children (Sutcliffe & Moss, 2010). Aurpibul et al. (2010) noted that between revaccination and 40 months post revaccination the children had not faced any serious illness or hospitalization. The situation in Thailand is quite different from Uganda and several African countries where children face at least three episodes of malaria in addition to malnutrition and other parasitic, viral, and bacterial infections in a year. These infections could contribute to waning of measles antibodies in HIV-infected children on HAART in Sub-Saharan Africa post vaccination (Fowlkes, et al., 2011; Kizito et al.,

2013; Tejiokem et al., 2007). The children in the Aurpibul et al. (2010) study were revaccinated at an average age of 9.6 years following immune recovery due to HAART that was started at about 7.7 years (Aurpibul et al., 2006). In measles highly endemic countries in Sub-Saharan Africa where children are exposed to and affected by measles mostly before 5 years (WHO, 2009a), revaccination at 9 years would be late; several children would have developed measles by this age, with some dying from the disease. Starting HAART within the first year of life, and considering revaccination at a much younger age when children are much more vulnerable to measles (below 5 years) may prevent such morbidity and mortality. However, the most appropriate age for revaccination needs to be ascertained as well as whether revaccination is necessary if HAART is started in the first year of life before measles vaccination.

Abzug et al. (2010) reported on two consecutive multicenter studies in the US conducted to investigate the effect of revaccination of HIV-infected children on HAART with immune recovery. Their initial study (P1024) evaluated immunogenicity of different vaccines, including measles, in HIV-infected children 2 to 19 years, in 35 sites in the US. Abzug et al. (2010) found that at entry into P1024, about 52% of the children had protective measles antibodies, a finding consistent with that by Aurpibul et al. (2006) who found about 60% of HIV-infected children on HAART had protective measles antibody. Viral load of less than 400 copies/ml and age of less than 7 years were associated with protective measles antibody levels at entry into the first study (P1024). Following revaccination, 89% had protective levels of measles antibodies by 8 weeks; the proportion reduced slightly to 80% by 80weeks, indicating that most of the revaccinated

HIV children on HAART had developed protective antibodies and maintained them for at least 20 months. This finding is consistent with the results of the Aurpibul et al. (2010) study. There were no statistically significant differences in the proportion with protective measles antibodies among the different strata based on level of immune recovery in all the post vaccination measurements in P1024. This finding implies that as long as there is immune recovery with CD4+ percentage greater or equal to 15%, revaccination may result in protection of the HIV-infected children on HAART (Abzug et al., 2010). Interestingly there was an inverse correlation between the HIV viral load and level of measles antibody at all post vaccination time points, with viral load of less than 400 copies per ml correlating with the highest antibody levels (Abzug et al., 2010). Even post revaccination, viral load of less than 400copies per ml was the only significant predictor of higher levels of measles antibodies. The authors indicate that it may be the HIV viral suppression or replication that influences the waning of measles antibodies and not the reduction in CD4+ percentage or absolute numbers. This contradicts the findings by Waibale et al. (1999) that indicated that it was not HIV infection per se, but malnutrition associated with the HIV infection that resulted in suboptimal measles vaccine response.

At entry into the follow up study from P1024 (P1061s), Abzug et al. (2010) found that 75% of the revaccinated participants in P1024 still had protective measles antibodies over 4 years after revaccination. This finding was similar that findings by Aurpibul et al. (2010) where 85% of revaccinated HIV-infected children on HAART, with immune recovery, developed and maintained protective measles antibodies for over 3 years. After the second revaccination in P1061s, Abzug et al. (2010) found that 83% of the

participants had protective levels of measles antibody at day 7 post revaccination, and 95% by day 28. However, only 5% had evidence of immunologic memory (equal to or greater than 4-fold increase in measles antibodies between entry and day 7 post vaccination) (Abzug et al., 2010). The presence of protective measles antibodies at day 7 post vaccination and immunologic memory were found inversely correlated to the viral load at entry into P1024 (Abzug et al., 2010) highlighting the role of HIV viral suppression (and not only increase in CD4+ percentage) in preserving protection against measles in HIV-infected children. Results of Abzug et al. (2010) imply that HAART may not prevent or adjust waning of measles antibodies or restore immunologic memory unless viral suppression is achieved early in HAART treatment and only if HAART is initiated when the immunity is not severely suppressed (Abzug et al., 2010). In addition, for best results, revaccination should be conducted when there is viral suppression. Sutcliffe and Moss (2010) explain that immune reconstitution in HIV-infected children on HAART involves generation of "naive T cells" and not expansion of memory cells. Thus, this may be the reason why HAART does not restore or adjust waning measles antibodies if vaccines were given and antibodies started waning before HAART initiation.

The strengths of Abzug et al. (2010) were the study design, longer duration of follow up, and the ability to do multiple tests including viral load at the different measurement points. The latter enabled better follow up of the participants. However, the sample size in the immunologic memory component was too small to allow for broad generalization. Abzug et al. (2010) conducted their study in a developed country setting;

it is not clear whether results would be similar in very low development settings like Sub-Saharan Africa. It is likely that even with HAART, the measles protective antibodies may be lower in Sub-Saharan Africa due to malnutrition and other infections and that lower levels of antibodies would be developed after revaccination, and or antibodies developed after revaccination may wane faster again, necessitating several booster doses. Although findings by Aurpibul et al. (2010) and Abzug et al. (2010) indicate that most HIV-infected children on HAART, with immune recovery, developed and maintained protective antibodies on revaccination, it is still not clear what the most appropriate age for initiation of HAART and for revaccination with measles vaccine in these children should be.

Summary

Several studies have been conducted to investigate the effect of HIV infection on children's response to the measles vaccine and all consistently indicate that HIV infection causes measles secondary vaccine failure (Aurpibul et al., 2006; Fowlkes, et al., 2011; Tejiokem et al., 2007; Waibale et al., 1999). One study indicated that there might also be primary vaccine failure in some HIV-infected children (Kizito et al., 2013). Some studies show that even when HIV-infected children are treated with HAART, the treatment does not prevent secondary vaccine failure regardless of age of initiation (Aurpibul et al., 2006; Sutcliffe & Moss, 2010). However, other studies show that initiation of HAART in the first year of life, before immune deterioration by HIV and before measles vaccination results in a vaccine response similar to that of HIV-uninfected individuals (Abzug et al., 2010; Pensieroso et al., 2009). However, comparing the study results was difficult

because the cut off for measles protective antibody level used varied in the different

studies. In addition, the sample size in most studies was small making generalization of

the findings beyond the study participants or beyond the country in which the study was

conducted difficult.

There is lack of consistency on the factors that could predict low measles

antibody in HIV-infected children (Sutcliffe & Moss, 2010). While some studies indicate

that HIV viral suppression, with a viral load below 400 copies/ml is a predictor of having

protective measles antibodies and not the CD4+ count, other studies indicate that the

CD4+ count could be a predictor of protective measles antibodies (Aurpibul et al., 2006;

Aurpibul et al., 2010; Sutcliffe & Moss, 2010; Tejiokem et al., 2007). Furthermore, one

study indicated that the age at initiation of HAART is a predictor for protective measles

antibodies (Pensieroso et al., 2009) while others indicated that it is not (Abzug et al.,

2010; Aurpibul et al., 2006). It has not been clearly demonstrated that the reduction or the

loss of measles antibodies implies loss of immunologic memory. However, given that

immune reconstitution following HAART produces naïve T cells and it does not involve

expansion of memory cells, it may be inferred that immune reconstitution may not restore

immunologic memory (Sutcliffe & Moss, 2010). Revaccination of HIV-infected children

with immune reconstitution has been conducted in some prospective studies and results

showed that most of the children developed and maintained protective measles antibodies

following revaccination. However, a few children still lost the antibodies and may require

more boosters to be protected from measles illness (Abzug et al., 2010; Aurpibul et al.,

2010; Melvin & Mohan, 2003). It is however inconclusive whether HIV-infected children

with unprotective measles antibodies experience a proportionately higher risk of measles illness compared to HIV-uninfected children. Furthermore, it is also not clear what the best age for revaccination of HIV-infected children on HAART should be to ensure these children maintain protective measles antibodies. Thus, monitoring measles antibody levels among children on HAART should become part of the care for HIV-infected children on HAART to identify timely children in need of revaccination (Sutcliffe & Moss, 2010).

Measles illness in HIV-infected children regardless of HAART is associated with higher rates of mortality than in HIV-uninfected children. Therefore, an appropriate vaccination strategy is necessary to ensure that HIV-infected children maintain protective levels of measles antibodies. It has been suggested that achieving herd immunity in the communities where the HIV-infected children live could protect them from measles, even if they have secondary vaccine failure (Helfand, Perry, & Strebel, 2007). Nevertheless, in most of Sub-Saharan Africa it is not yet feasible to achieve herd immunity in all communities where there is high prevalence of HIV. It remains pertinent to develop vaccination guidelines in Sub-Saharan Africa to ensure protection of each HIV-infected child. My study, guided by the proximate determinant framework, generated information on the risk of measles illness in HIV-infected children on HAART, possible predictors of the risk, and on the appropriate age for revaccination to ensure protection of the HIV-infected children from measles. In Chapter 3, I describe the methodology used for the study and present the findings in Chapter 4.

Chapter 3: Research Method

Introduction

The main purpose of this study was to examine whether there is a difference between the risk of measles in HIV-infected children on HAART and that of HIV-uninfected children of the same age group. I also investigated the relationship between nutritional status (undernutrition), age at initiation of HAART, low CD4+ count, and the risk of measles among HIV-infected children on HAART. Lastly, I assessed the age group most affected by measles among HIV-infected children on HAART to determine the appropriate timing for revaccination, if necessary.

In this chapter, I describe the proposed methodology for this quantitative study including the research design and rationale, study population, sample size and power obtained, participants' enrolment procedures, data collection instrument, list of variables and ethical considerations. I also highlight the data management plan, threats to validity, and measurement and how they were controlled.

Research Design and Rationale

A retrospective cohort design was used to answer the primary and secondary research questions. The retrospective cohort study design was appropriate for this study because it enabled comparison of the risk for measles in HIV-infected children on HAART (exposed children) with that in HIV-uninfected children (unexposed) of the same age group, from the same residential districts that experienced outbreaks of measles (Mann, 2003; Song & Chung, 2010).

A prospective cohort study could have been used to follow the HIV-infected and uninfected children from 6 weeks when HIV infection is confirmed or ruled out, through measles vaccination. The children would be followed through initiation of HAART in HIV-infected children and then compare incidence of measles in the HIV-infected children on HAART and HIV-uninfected children. However, this would have required that children were enrolled at approximately 6 weeks of age and followed for several years. It also would have required substantial financial, human, and material resources for regular follow up (Mann, 2003; Song & Chung, 2010).

Identifying and maintaining contact with children with loss of protective measles antibodies to ascertain if they will develop measles disease when exposed to wild measles virus may be unethical as it would involve following children with known measles susceptibility to watch them develop the disease I used a retrospective cohort study design for this study. All of the required information was collected after disease occurrence, which eliminated the need for prospective follow up. A retrospective cohort study is cheaper than a prospective cohort and can be completed within a shorter timeframe (Mann, 2003; Song & Chung, 2010). To answer the secondary questions, I assessed the relationship between nutritional status before measles illness, low CD4+ count before measles illness, and age at initiation of HAART and measles illness. The aim of the assessment was to ascertain if any of these factors was a significant predictor of risk of measles in HIV-infected children on HAART.

For the primary question, the dependent variable (outcome) was measles illness while the main independent variable (exposure) was HIV infection and use of HAART.

Measles illness for this study was defined as measles infection recorded in medical records based on a clinical diagnosis by a clinician, with or without laboratory confirmation. During measles outbreaks in Uganda, after laboratory confirmation of the outbreak by positive measles IgM test results for 5 to 10 cases in a district, the other measles cases are considered epidemiologically linked and clinically diagnosed. Hence, in this study, a measles diagnosis was based on a clinical diagnosis as seen in medical records of the children in the study. Categorization of the exposure status was based on medical records indicating a positive HIV test result and HAART treatment for children who were 2 to 15 years old and residents in the Kampala and Wakiso districts in November 2011. For unexposed children, categorization was based on the medical records not indicating positive HIV test results and/or indicating HIV negative results for children of the same age group resident in Kampala and Wakiso district by November 2011.

To answer the secondary questions, the analysis focused on the HIV-infected children on HAART only. The dependent variable was measles illness and the independent variables were nutritional status, CD4+ count, and age at initiation of HAART. Measles illness was defined as measles recorded in medical records. The CD4+ count and percentage were obtained from the measurements of these variables recorded at the child's last clinic visit prior to measles illness or measles outbreak, within 3 to 6 months. When the CD4+ percentage was not indicated in the records, it was manually computed by dividing the CD4+ count by the total lymphocyte count indicated in the full blood count conducted on the same day as the CD4+ count. The CD4+ count/percentage

was categorized into low CD4+ count (less than 25%) and normal CD4+ count (25% and above). The age at initiation of HAART was the age (years) at which the medical record indicated that a child had been started on HAART. Nutritional status was based on the weight and height measurements of the child recorded at the last clinic visit prior to measles illness or measles outbreak within 3 to 6 months. Nutritional status was categorized into normal nutrition status or malnutrition (undernutrition) based on the height and weight measurements of the child recorded at the last clinic visit prior to measles illness and on the recommended cut offs for the above categories by World Health Organization (WHO, 2006a). Although age at initiation of HAART was collected and analyzed as a continuous variable, it was further categorized during analysis into initiation of HAART at age less than 9 months and initiation at 9 months of age or more. The purpose of this age categorization was to assess if starting HAART at an early age, before measles vaccination, was a predictor of risk of measles. In Figures 2 and 3, I display the approach to the primary and secondary questions respectively.
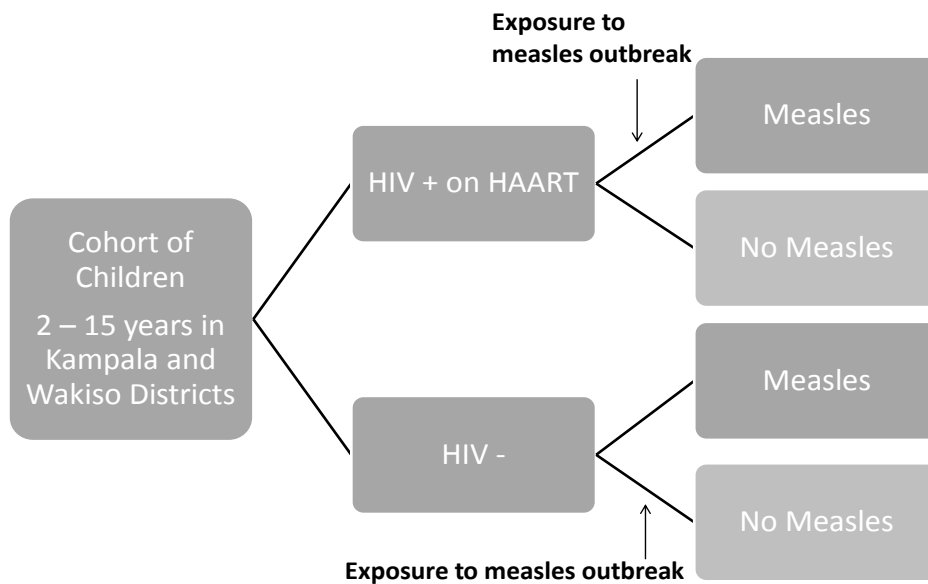
Figure 2. Schematic display of the approach to the study primary question
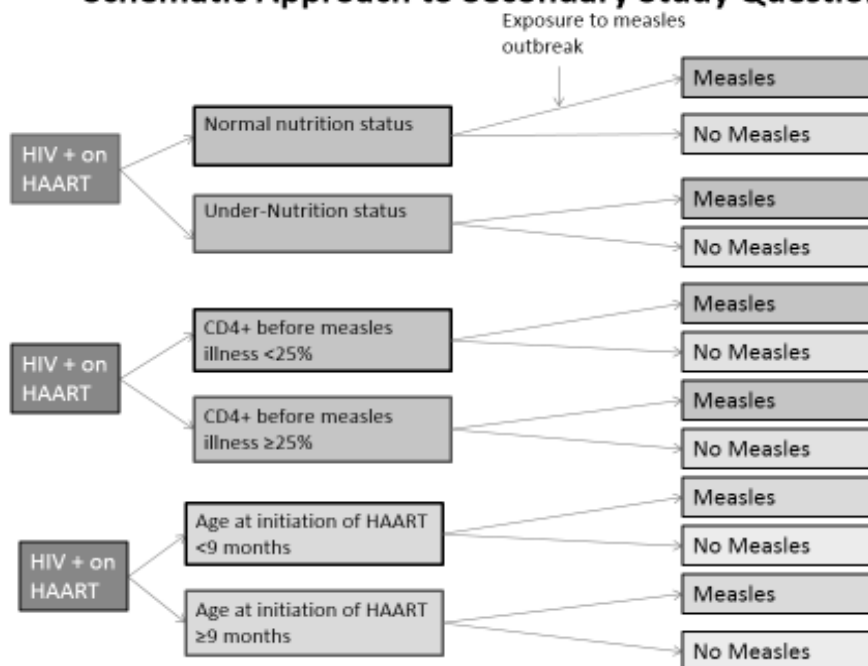
Figure 3. Schematic display of approach to study secondary questions

Study Site and Population

The study site was the Nsambya Hospital, the 2nd largest hospital in the city of Kampala, Uganda. This hospital's catchment population is about 4,000,000 people from the districts of Kampala, Wakiso, Mpigi, and Mukono (Massavon, Barlow-Mosha, et al., 2014; Massavon, Mugenyi, et al., 2014). Fifty one percent of this population are children less than 15 years old (Associazione Italiana per la Solidarietà tra i Popoli [AISPO], n.d; Uganda Bureau of Statistics [UBOS], n.d). The Nsambya hospital handles approximately 320,000 admissions per year and three times as many outpatient consultations (AISPO, n.d). The hospital is involved in clinical and public health research and is a training institution for medical internships and graduate studies in clinical disciplines of medicine (East African Consortium for Clinical Research, 2010; Massavon, Mugenyi, et al., 2014).

The study sample of exposed children was drawn from the home care department (HCD), which provides care for children and adults infected with HIV, including initiation and monitoring of HAART, among other services. The sample of un-exposed children was drawn from the Out-Patient Department (OPD) of the hospital where all patients (except HIV-infected clients) are first seen upon arrival at the hospital. The HCD provides care to about 14,000 HIV-infected clients, of which about 8.4% are children (Massavon, Mugenyi, et al., 2014).

By April 2013, 12,899 HIV patients were under care at Nsambya Home Care, including 856 children less than 15 years old. Forty nine percent of the HIV patients were on HAART, including 395 children less than 15 years old and 380 children 2 to 15 years old (Nsambya Home Care, 2013). Given the number of HIV-infected children on

HAART seen at the HCD, and the large number of pediatric OPD consultations, selection of a representative sample of children was feasible in this site.

Selection of Exposed and Unexposed Children

From the cohort of children 2 to 15 years old in Wakiso and Kampala districts, exposed children (who were HIV-infected and on HAART) were selected from pediatric patients seen at Nsambya hospital HCD from November 2011 to June 2012, a period during which a measles outbreak was experienced in Kampala and Wakiso districts. The measles outbreak was confirmed by the measles laboratory at Uganda Virus Research Institute in November 2011 and monitored weekly through the measles surveillance system (Mwesigye, 2012). Through the weekly surveillance system, it was noted that the outbreak ended in June 2012 (Ministry of Health Database (DHIS2). Unexposed children (HIV-uninfected) were selected from children 2 to 15 years old who attended the OPD at Nsambya hospital during the same period.

Inclusion Criteria

The inclusion criteria included HIV-infected children on HAART before November 2011 as the exposed and HIV-uninfected as the unexposed children; all children were 2 to 15 years old before November 2011 and residing in Kampala or Wakiso districts during the time period of November 2011 to June 2012 when Kampala and Wakiso districts experienced a measles outbreak. For the exposed children, HIV diagnosis should have been confirmed and HAART initiated before the measles outbreak period (November 2011 to June 2012). For the unexposed children, there should have

been no evidence of HIV positivity from the medical records (no mention of HIV

positivity in medical records or presence of HIV negative results in the medical records).

Sample Size and Power

A Priori Sample Size Estimation

To determine the minimum sample size needed for this study, I used a formula for

sample size derivation for cohort studies (Hajian-Tilaki, 2011; Kasiulevicius, Sapoka, &

Filipaviciute, 2006). The key issues for consideration in estimating the sample size a

priori were: (a) the type 1 error (α), which was set at 0.05 (5%); (b) the desired power of

the study, which was 80%; (c) the probability of disease in the unexposed group (P_0),

estimated at 25% (Mupere et al., 2006); (d) the estimated probability of disease in the

exposed group (P_1), estimated at 40% (Fowlkes et al., 2011; Tejiokem et al., 2007); and

(e) the minimum relative risk desired to be detected (1.6), which was derived from

dividing P_1 by P_0 (0.4/0.25). The selection of a P_1 of 40% was based on studies that

indicated that by 2 to 3 years about 60 to 80% of HIV-infected children do not have

protective measles antibodies and are susceptible to measles (Fowlkes et al., 2011;

Tejiokem et al., 2007). An increase of 55% in children without protective antibodies

among HIV-infected children compared to the general population of children (80%

versus 25%) was estimated (Fowlkes et al., 2011; Mupere et al., 2006; Tejiokem et al.,

2007). Additionally, a 60% increase in probability of measles in the exposed was

estimated, hence the estimated probability of measles in the exposed of 40% (computed

by increasing the P_0 of 25% by 60%). The parameters considered in estimating the

sample size a priori are summarized in Table 1 below.

Table 1

Parameters Considered in Estimating the Sample Size a Priori

Parameter	Value Used in a Priori Sample Size
Probability of Measles in the Exposed (P_1)	40%
Probability of Measles in the Unexposed (P_0)	25%
Relative Risk	1.6
Type 1 Error (α)	0.05
Type 2 Error (β)	80%

The a priori estimated minimum sample size was 152 children for each group. An additional 10% of participants were added to account for possible dropouts from analysis due to missing information. Thus, a sample of 168 HIV-infected children who were on HAART and 2 to 15 years old by November 2011 was required for this study. The unexposed group was an equal number of HIV-uninfected children of the same age group at the same time.

Post-hoc Power Analysis

Among study participants, the proportion of exposed children that developed measles was 5.7%, which was lower than the a priori selected P_1. Similarly, the proportion of un-exposed children seen at the OPD that developed measles during the measles outbreak period was lower than the a priori selected P0 (4% versus 25%). The

assumption behind the apriori P_0 and P_1 estimation was that the children without protective measles antibodies, approximately 40% of the exposed and 25% of unexposed (Fowlkes et al., 2011; Mupere et al., 2006; Tejiokem et al., 2007), would be susceptible to and could suffer from measles when exposed to the wild measles virus during the outbreak. This assumption did not hold for this study. With the differences in P_0 and P_1 encountered, and keeping the desired odds ratio of 1.6, a post-hoc study power was calculated at about 20% (Epi Info 7 StatCalc).

Selection of Exposed Cohort of HIV-infected Children on HAART

The HCD keeps a computerized database that includes information on all their HIV clients including name, age, district of residence, enrollment number, date of enrollment at the clinic, date started on HAART, dates of follow up visits and results of some tests taken on the follow-up visits. Although, this database had most clinical information, laboratory results, especially CD4+ results, were missing for some children. The HCD also has a separate database with more detailed information on HAART regimen and monitoring, where clients are identified by their names and HAART number. These two databases are linked by the enrollment number. The two databases were used to create a list of exposed children that were diagnosed as HIV-infected and started on HAART by November 2011 and in care at the department. Then the required study sample was randomly selected from this list using a table of random numbers. Every child was given a 3-digit serial number, which became the study participant's unique identifier. In the interest of having as high power for the study as possible, I selected 223 exposed children for this study.

Selection of Unexposed Cohort of HIV-uninfected Children

The Nsambya hospital electronic OPD database was accessed to generate a list of children 2 to 15 years old who attended the OPD between November 2011 and June 2012. From this list, children residing in districts other than Kampala and Wakiso were discarded, as well as children who were HIV positive. Only children with no evidence of being HIV-infected were included among the unexposed. From the remaining children on the list, 223 children were randomly selected using a table of random numbers.

Enrollment Procedures

After receiving the approvals to start the study from the Institutional Review Boards of Walden University, Nsambya hospital, The AIDS Support Organization (TASO), and Uganda National Council of Science and Technology, and permission from Nsambya Hospital to access the required medical records for data collection, meetings were conducted with the Nsambya HCD, OPD and the Medical Records staff. During these meetings, the study questions and enrollment procedures were discussed and staff cooperation requested. A person was assigned in HCD and Medical Records to work with me to identify and retrieve the medical charts needed one by one. Thereafter, the selection of children was conducted in collaboration with the HCD data manager and head of medical records department for exposed and unexposed children respectively.

Data Collection

Data Collection Instrument

A data collection instrument was developed for the study to record data collected from the study participants' medical records. A copy of this data collection tool is

included in the Appendix. The data collection tool was shared with one national expert in

measles and one national expert in HIV, as well as my dissertation committee for

assessment of content validity. After enrollment, the medical records of 10 randomly

selected study participants (5 exposed and 5 unexposed children) were reviewed twice on

different days and data retrieved in the second review compared with that collected in the

first review to assess validity of the data collection tool as well as reliability of data.

Data Collection Procedure

After selection of the exposed and unexposed study participants, I accessed the

electronic database and the hard copy of the medical records of the selected exposed

participants to extract required data. As the study involved review of records, with no

direct contact with any of the children in the study or their guardians, informed consent

was not necessary. I retrieved data on the exposed children from both the electronic and

hard copy medical records at HCD. From the general HCD electronic database, I

extracted the following data on the selected children: (a) enrollment number, (b) the date

when the child enrolled into care at Nsambya HCD, (c) the date of birth (which was only

used to compute age by November 1st, 2011 and Z-scores and not included in study

database); (d) sex, (e) date of HAART initiation, (f) CD4+ count at HAART initiation

and (g) the date the CD4+count was assessed for some of the children. Using the

enrollment numbers, the HCD data manager assisted me to identify from the HAART

database the corresponding HAART numbers. Using the HAART numbers, the hard copy

medical records of the selected children were retrieved (20 at a time) from the archive

and stored in the medical records office at Nsambya HCD. I was given a room within the

HCD, near the medical records office, to conduct the data extraction. I accessed and reviewed the hard copy medical records one by one to manually collect the information on districts, sub counties, villages, immunization status, date of HAART initiation, CD4+ count and percent at HAART initiation. I also collected information on history of measles, age at measles for those that suffered from measles, CD4+ count and percent, weight, and height prior to measles for the children who had a history of measles. Other variables on which I collected information include the latest CD4+ count and percent, weight and height prior to November 1st 2011 for the children that did not suffer from measles. For children with history of measles illness, the date of illness was extracted and used to ascertain that illness occurred during the measles outbreak period or before and to compute age at measles illness. Data on clinical presentations and measles complications were also collected to ensure that the clinical diagnosis was based on an accepted case definition.

Data on some variables were missing from medical records and thus could not be extracted. For example in some records for exposed children, CD4+ count at HAART initiation was recorded but not the corresponding CD4+ percentage. Instead, what was indicated was the normal CD4+ range against which one could tell that the CD4+ count was below normal or within normal range. Similarly, CD4+ count prior to measles for some exposed children that suffered from measles, and CD4+ prior to November 1st 2011 for some children that did not suffer from measles was indicated with the normal range but without the corresponding percentage. Immunization status on enrollment into care at Nsambya HCD was indicated for some of the exposed children but not others.

Furthermore, immunization status was assessed based on the routine immunization schedule only. The records indicated if the children had received measles vaccination as part of the routine immunization schedule or not; thus the number of doses recorded for those that had received measles vaccination was one (1). There was no record or question in the enrollment or follow up tools on measles vaccine doses from mass measles vaccination campaigns. Some children (14) that did not suffer from measles between November 2011 and June 2012 had already suffered from measles illness prior to November 2011 as indicated in their medical records. Data on the age at measles, CD4+ count and percentage, weight and height prior to measles illness were also extracted for use in assessment of the age group most affected by measles in secondary RQ 1 and the risk factors for measles illness among the HIV-infected children on HAART in secondary RQ 2.

Data from the unexposed participants were retrieved from the electronic and hard copy medical records of children that attended Nsambya General Outpatient Department (OPD) between November 2011 and June 2012. From the electronic OPD database, data extracted for the selected unexposed children included: Clinic number, age, sex, district, village, and diagnosis. This database lacked information on immunization status and sub-county of residence of all the children. Thus, I used the Land Conflict Mapping Tool (LCMT) (LCMT, 2015) to identify the sub-counties of residence for all the unexposed study participants. In addition to the electronic database, there was a hardcopy register of all children seen in OPD that were referred for admission to the pediatric ward. This register had more details on the admitted cases including sub-county of residence, final

diagnoses, and immunization status. Thus, immunization status of the unexposed could only be ascertained for a proportion of the selected children seen at OPD that were later admitted to the pediatric ward. Even then, immunization history was based on the routine immunization schedule and no mention was made of any doses received from mass measles vaccination campaigns. Hence, for those that were vaccinated, the number of doses was one (1). For children with history of measles illness that were admitted, data on clinical presentations and complications was extracted from the hard copy register to ensure that the clinical diagnosis was based on the accepted case definition. When data extraction was conducted, a study number was assigned for each enrolled child and indicated on the data collection tool; no person identifiers were included. Thus the data was de-identified after the data extraction stage and information entered in the database was anonymous.

Data Processing

  Data Entry. A data entry screen matching the study data collection tool was developed in Epi Info 7.0 (Centers for Disease Prevention and Control, n.d.). Checks were included in the data entry screen to minimize errors. I entered twice all the epidemiological and clinical data into the Epi Info tool on a weekly basis. At the end of the data collection, data cleaning was undertaken before analysis was conducted. Data analysis was conducted using SPSS version 21 (Softonic, 2014).

  Data Cleaning. After data entry, each variable was reviewed in the two datasets obtained from the double entry. Where differences were found, reference was made to the original data collection tool to correct discrepancies. Additionally, frequency

distributions were obtained for each variable in both datasets. Where discrepancies,

unexpected or codes outside the designated codes were found, reference was made to the

original data collection tool to correct them. In the last step of data cleaning, I analyzed

variables that were related to ascertain if there was coherence in the data. For example,

unexposed children should have no data against the variable "age at initiation of

HAART", etc. If incoherencies were detected for some entries in both data sets, reference

would be made to the hard copies for correction of the data entry errors.

Data Transformation

In some instances, where the CD4+ percentage was not indicated, total

lymphocyte count was indicated as part of a full blood count conducted on the same day

as the CD4+ count. In such cases, I computed the CD4+ percentage manually by dividing

the CD4+ count by the total lymphocyte count and then multiplying by 100. The CD4+

percentage before measles illness or outbreak was categorized into less than 25%

(immune suppression) and 25% and above (normal immunity) during data analysis. The

CD4+ percentage prior to initiation of HAART was categorized into less than 15%

(severely immune suppressed) and 15% and above (not severely immune suppressed. The

age at initiation of HAART was categorized into less than 9 months and 9 months and

above in the analysis. Duration on HAART was computed by subtracting the age at

initiation of HAART from the age at exposure to measles outbreak. The duration on

HAART was further categorized into less than 2 years and 2 years and above during

analysis. The data on weight and height was transformed into Z-scores to enable

categorization of nutrition status into normal or under nutrition. Z-scores were computed

for weight for age, height for age, and weight for height (or Body Mass Index (BMI) for age for children above 5 years) using the WHO Anthro software. WHO AnthroPlus software was used to compute the Z-scores for children above 5 years respectively (WHO, 2009; WHO, 2014a). The date of measurements, the date of birth, the weight (in kilograms) and the height (in centimeters), were entered into the respective field in the software. Furthermore, I indicated in the relevant field in the tool whether the height was taken when the child was standing or lying down, and then the tool automatically computed the Z-scores for the child. Using the WHO predetermined cut offs for Z-scores (WHO, 2006a), the Z-score was used to categorize nutrition status as either normal nutrition status (Z-score less than 2 and more than -2) or undernutrition (Z score less than -2) in the analysis. When computing the Z-scores, for children older than 5 years, the WHO AnthroPlus software would generate BMI for age Z-Score instead of weight for height Z-score. Furthermore, when children were above 10 years of age, the software would indicate "NA" (not applicable) for weight for age Z-score because it is designed to compute weight for age up to 120 months (WHO, 2009).

List of Variables and Levels of Measurement

In Appendix 2 the variables that were used to answer the different study questions, and their description are indicated. The codes used in the database and the level of measurement for the different variables are shown in Appendix 3. The data analysis plan in Table 1 indicates how the different variables were used in the data analysis.

Data Analysis Plan

Table 2 below shows a summary of the data analysis plan. Details of how descriptive epidemiology, bivariate and multivariate analysis were conducted are indicated in the respective sections below. Furthermore, in this section, I describe the analysis conducted for each research question.

Table 2

Summary of Data Analysis Plan

Research Question	Sample	Variables	Analysis Plan and Statistical test(s)
Descriptive Epidemiology	223 exposed and 223 unexposed children	Age, sex, vaccination status, number of vaccine doses, age at measles illness	Mean, median, and range of age of participants, sex distribution; age at measles illness Comparing the above in exposed versus unexposed children
Descriptive Epidemiology	223 exposed children	Age, Age at HAART Initiation, CD4+ count at HAART initiation, CD4+ percentage at HAART initiation, duration of HAART, CD4+ count and CD4+ percentage prior to measles illness or outbreak, age at measles, Under Nutrition on Height for Age (Stunting), Nutrition on Weight for Age (Underweight), Nutrition on Weight for Height (Wasting) Measles illness among the exposed	Mean, median, and range of the different continuous variables Comparison of the means of the different variables in exposed children who suffered from measles versus those that did not suffer from measles Comparison of the proportions of stunting, underweight, and wasting in exposed children who suffered from measles versus those that did not suffer from measles Age distribution of measles cases among the exposed

Research Question	Sample	Variables	Analysis Plan and Statistical test(s)
Primary study question	223 exposed and 223 unexposed children	Measles illness in exposed and unexposed children (dependent variable) Independent variables: Age and sex were variables adjusted for	Relative Risk Risk difference Cohen's h Bivariate analysis Multiple logistic regression Post-hoc power analysis using obtained P_0 and P_1.
Secondary question – Age at HAART Initiation	223 exposed children	Dependent variable: Measles illness in exposed children Age at HAART initiation, duration of HAART, Age at exposure to measles, sex, CD4+ percentage prior to HAART, and CD4+ percentage prior to measles illness or outbreak	Bivariate Analysis Multivariate analysis
Secondary question – Nutrition Status prior to measles	223 exposed children	Measles illness in exposed children (dependent variable) Weight and height prior to measles illness	Proportions of children with under nutrition by category (weight for age, BMI for age, and height for age) among the exposed Bivariate analysis using the different categories of malnutrition (Underweight, wasting, and stunting)

Research Question	Sample	Variables	Analysis Plan and Statistical test(s)
Secondary question – Nutrition Status prior to measles	223 exposed children	Independent variables: Under nutrition for weight for age (Underweight); Under nutrition for weight for height/BMI for age (wasting); Under nutrition for height for age (stunting); age at exposure to measles; sex; age at initiation of HAART; CD4+ percentage prior to HAART; and CD4+ percentage prior to measles illness or outbreak	Multivariate analysis
Secondary question – CD4+ percentage prior to measles or measles outbreak	223 exposed children	Dependent variable: Measles illness in exposed children Independent variables: CD4+ percentage before measles illness or outbreak; CD4+ prior to initiation of HAART, and duration on HAART age, sex	Proportion of exposed children with CD4+ percentage less than 25% prior to measles Mean CD4+ percentage at HAART initiation Bivariate analysis Multivariate analysis

Research Question	Sample	Variables	Analysis Plan and Statistical test(s)
Secondary question – Nutrition status prior to measles, Age at HAART Initiation, and CD4+ percentage prior to measles	223 exposed children	Dependent variable: Measles illness in exposed children Independent variables: Under Nutrition on Weight for age (Underweight), Under Nutrition on Height of age (Stunting), Under Nutrition on BMI for age (Wasting), Age at HAART initiation, CD4+ percentage prior to measles illness or outbreak, Age, sex	Bivariate analysis Multivariate analysis

Descriptive Epidemiology

I conducted univariate analysis for the descriptive epidemiological analysis of study participants. This descriptive analysis included the participants' mean and median age at time of measles outbreak (November 2011 to June 2012) as well as the age range, sex distribution, and vaccination status by group (exposed and unexposed). I also conducted an independent sample T-test to compare the mean of age of exposed and unexposed children. For the exposed children only, I obtained the mean age at HAART initiation, mean CD4+ at HAART initiation, mean CD4+ percentage prior to initiation of HAART, mean CD4+ prior to measles, mean CD4+ percentage before measles illness or outbreak, and mean duration on HAART. I compared the above parameters for exposed children who suffered from measles and those who did not. In addition, I analyzed the proportion of exposed children that were underweight, stunted, or wasted, and compared the proportions among those that suffered from measles and those that did not. The proportion of exposed and unexposed children that developed measles illness was computed. The age distribution of measles cases among the exposed was analyzed to provide information on the most affected age group by measles among the HIV-infected children on HAART. The age distribution of measles cases was displayed in form of a histogram.

Bivariate and Multivariate Analysis

Bivariate analysis was conducted for the main independent variables (age at initiation of HAART, CD4+ percentage before measles or measles illness, and nutrition status (stunting, wasting, and underweight), and for each of the other possible covariates

that were identified for each question and measles illness as the dependent variable.

Thereafter, I conducted forward stepwise binomial logistic regression, adding one

variable at a time to the main independent variable for each question in the regression

model. If a covariate added was found to be a significant predictor of measles (with p

equal to or less than 0.05), interaction between the variable and the main independent

variable for the question was assessed using the chunk test. If there were no interaction

between the variable and the main independent variable for the question, then the variable

would be left in the model. If the variable was not significant, possible confounding

effect of the variable added on the relationship between the main independent variable for

the question and measles was assessed.

A cut off of 10% change between the crude and adjusted odds ratio was used to

decide whether the variable was a confounder of the relationship or not. If the change was

10% and above, the variable was considered a confounder and less than 10% indicated

that the variable was not a confounder. If a variable was found to be a confounder, this

too was left in the model. To rule out collinearity of the variables included in the model, I

conducted simple linear regressions with different combinations of the variables to be

included in the different models. I then observed the tolerance, variance inflation factor

(VIF), standard error of the beta coefficient, and square of R (R^2). If R^2 was higher than

0.9, the tolerance ($1 - R^2$) was less than 0.1, the standard error of the beta coefficient was

higher than 2, and VIF was higher than 3, I would consider that there was collinearity

between the independent valuables involved and not include those variables in the same

model. The final model included the main independent variable for the question, any of

the other variables that were found to be significant predictors of the outcome as well as any variables that were found to be confounders of the relationship between the main independent variable for the question and measles.

At each step the model fitness and variance as shown by Hosmer and Lemeshow Test and Nagelkerke PseudoR^2 respectively were noted. The final model had the highest model fitness percentage. The Nagelkerke PseudoR^2 is a measure of the percentage of variance in the outcome explained by the model. The odds ratio of the main independent variable of the question in the final model was considered the adjusted odds ratio and statistical significance ascertained from its corresponding *p* value. However, where the number of valid cases in the analysis could not satisfy the ratio of 20 cases to one variable in the model, instead of stepwise regression, I used simultaneous logistic regression. In simultaneous regression, after assessing for confounding and interaction as indicated above, I included all the relevant variables in the model at the same time. Then I looked at the standard errors for the coefficient *β* for each variable to rule out collinearity. I applied simultaneous logistic regression only in the analysis of the relationship between underweight and measles illness in the exposed children.

Primary Question

To answer the primary study question, I computed the relative risk (RR) of measles among HIV-infected children on HAART compared with HIV-uninfected. I used a 2 by 2 contingency table to ascertain the relative risk. Cohen's *h* was computed to describe the difference between the obtained P_0 and P_1 and ascertain whether the difference was meaningful. If Cohen's *h* is 0.2, the difference was considered small; if *h*

is 0.5, the difference was moderate, and if *h* is 0.8, the difference was large. Effect size above 0.2 was considered meaningful and worth discussion (Mitchell, 2015).

I conducted bivariate analysis to ascertain if individually the age and sex were significantly associated with measles illness. A contingency 2 by 2 table was also used to ascertain the risk ratio for sex. However, the contingency 2 by 2 table could not be used for age at time of measles as this was used as a continuous variable. Thereafter, forward stepwise binomial logistic regression was conducted as described above to adjust for possible confounding effects of age and sex. Given that P_1 and P_0 were less than 10%, the odds ratio generated from logistic regression was approximately the same as the adjusted relative risk (McNutt, Wu, Xue, & Hafner, 2003). Adjustment for vaccination status and number of vaccine doses received could not be conducted due to missing information on this for most of the unexposed children. The Chi-Square test was used to ascertain the statistical significance of the difference in the relative risk of measles between the exposed and unexposed children. Cohen's *h* was used to assess if the difference proportion of measles in exposed and un-exposed was meaningful. Furthermore, the confidence intervals of the risk difference were reviewed to ascertain if the difference was significant or not. A confidence interval including zero is indicative of a non-significant difference (Scott, 2008). Based on the results of the above analysis, a decision was made whether to reject or not reject the null hypothesis related to the primary question.

Secondary Questions

Age at Initiation of HAART. To examine whether age at initiation of HAART was a significant predictor of risk of measles in HIV-infected children on HAART, I compared the proportion of HIV-infected children initiated on HAART before 9 months that developed measles with that of children who started on HAART at 9 months and above who developed measles. I conducted cross tabulation of age at initiation of HAART (less than 9 months; 9 months and above) and measles illness. To understand further the relationship of HAART on risk of measles, I analyzed for the possible effect of duration on HAART on the risk of measles. Given the very low number of children (3) who had been on HAART for less than 6 months, duration on HAART (less than 6 months/6 months and above) was excluded from the analysis. Instead, duration on HAART for less than 2 years was compared with duration of 2 years and above. I cross-tabulated duration on HAART (less than 2 years; 2 years and above) and measles illness. I then conducted bivariate analysis for age at initiation of HAART, age at exposure to measles, sex, duration on HAART, CD4+ percentage prior to HAART, and CD4+ percentage prior to measles illness or measles outbreak as independent variables and measles illness as the dependent variable. I further conducted forward stepwise binomial logistic regression as described above to ascertain if age at exposure to measles, sex, duration on HAART, CD4+ percentage prior to HAART, CD4+ percentage prior to measles illness or outbreak influence the relationship between age at initiation of HAART and risk of measles. Based on the p-value of the odds ratio of age at initiation of HAART in the final model, I made inference on whether the variable was a significant

predictor of measles in HIV-infected children on HAART and the null hypothesis was rejected or not rejected.

Nutrition Status. Height for age is a measure of linear growth and can be used to assess chronic malnutrition (stunting) in children (Shetty, P., n.d.). Hence the children that had under nutrition for height for age were stunted. Weight for age is a measure of growth in relation to chronological age and the children with under nutrition for weight for age are underweight (Shetty, P., n.d). The Body Mass Index (BMI) is a measure of the weight of an individual in relation to his/her height and indicates whether an individual is wasted or not (Shetty, P., n.d). Thus, children with under nutrition for weight for height or BMI are wasted. The terms stunted, underweight, and wasted were used in reporting of results. Stunting is an indication of chronic malnutrition while wasting is an indication of recent or acute malnutrition (Shetty, n.d). Being underweight is an indication of disharmony between the linear growth and body composition in reference to age (Shetty, n.d). Each of these types of malnutrition could affect the risk of measles differently in HIV-infected children (Waibale et al., 1999; Korutaro et al., 2013). Hence, in this study, I assessed if each of them was a predictor of measles separately. I also assessed for collinearity between these three parameters of under nutrition as indicated above. As I found no collinearity between them, I used each of them as an independent variable in the analysis.

To ascertain whether under nutrition was a significant predictor of the risk of measles in HIV-infected children on HAART, proportions of children with normal and undernutrition prior to measles (using the weight for age, height for age, and BMI for age

parameters separately) that developed measles were computed. To generate Z-scores for weight for age, height for age, and weight for height, the weight and height measurements, date of the measurements, and date of birth for children less than 5 years and 5 years and above respectively were entered into the WHO Anthro and WHO AnthroPlus software. Using the WHO predetermined cut-offs (WHO, 2006a), the Z-scores weight for age, weight for height, and height for age were used to categorize the nutrition status of a child. If the Z-score was less than -2, then the nutrition status was categorized as under nutrition. When the Z-score was more than -2, the nutrition status was categorized as normal nutrition. For children less than 5 years, the WHO Anthro software computed the Z-score for weight for height; however for children 5 years and older, the WHO/AnthroPlus software computed the Z-score for Body Mass Index (BMI) for age instead of weight for height. As there were very few children less than 5 years, the entries for weight for height were very few and this was excluded from the analysis. Instead, the analysis for weight for height parameter used BMI for age. In computing Z-score for weight for age, the software indicated "Not Applicable" (NA) if the age of the child exceeded the 10 years (120 months) (WHO, 2009). Hence, for some exposed children there were no Z-scores for weight for age. Z-scores for Height for Age and BMI for age were available for many of the exposed children.

Height for age. I cross tabulated the height for age prior to measles illness or outbreak and measles illness. I conducted bivariate analysis with height for age, age at time of exposure to measles, sex, CD4+ percentage prior to HAART, and CD4+ percentage prior to measles illness or outbreak as the independent variables and measles

illness as the dependent variable. Stepwise binomial logistic regression was conducted,

adding one variable to under nutrition (stunting) in the regression model at a time. Age in

years was first added to the model as it had the lowest *p* value on bivariate analysis. From

the model, age was a significant predictor of measles with a *p* value of 0.05 and was

retained in the model together with stunting. I assessed for interaction between age in

years and stunting using the chunk test and found that age was not interacting with

stunting. In the next step, CD4+ percentage prior to HAART was included in the model,

and it was not a significant predictor of the relationship between stunting and measles.

CD4+ percentage prior to HAART was then assessed for confounding at 10% cut off, and

found to be a confounder of the relationship between stunting and measles. CD4+

percentage prior to HAART was thus also retained in the model. In the next step, I

included sex in the model, and found that it was not a significant predictor of measles and

not a confounder of the relationship between stunting and measles; so I dropped sex from

the model. In the next step, I included CD4+ percentage prior to measles or measles

outbreak, and found that it was not a significant predictor of measles but was a

confounder of the relationship between stunting and measles; I thus left it in the model.

Hence, the final model included stunting, age at time of exposure to measles (in years),

CD4+ percentage prior to HAART, CD4+ percentage prior to measles illness or

outbreak. The adjusted odds ratio of stunting in the final model was considered the

adjusted odds ratio for stunting. At each step, the model fitness and variance were

indicated by the corresponding Hosmer and Lemeshow Test and Nagelkerke PseudoR2

respectively. Based on the adjusted odds ratio and *p* value from the final model, I inferred

on whether under nutrition for height for age (stunting) was a significant predictor of measles illness in HIV-infected children on HAART and the hypothesis rejected or not rejected.

Weight for Age. Cross tabulation was conducted for the weight for age prior to measles illness or outbreak measles illness. I conducted bivariate analysis for under nutrition for weight for age (underweight), age at exposure to measles, sex, CD4+ percentage prior to HAART initiation, CD4+ percentage prior to measles illness or outbreak as independent variables and measles illness as the dependent variable to ascertain statistical significance of any the variables. I then conducted multivariate analysis (simultaneous binomial logistic regression), adding all variables at a go to underweight in the model. The independent variables were also assessed for a confounding, interaction effect, or collinearity on the relationship between underweight and measles illness individually before putting them into the model. The odds ratio of underweight and the corresponding *p* value in the final model were used to infer whether underweight was a significant predictor of measles illness in HIV-infected children on HAART. Based on the result in the final model, the null hypothesis was either rejected or not rejected.

BMI for Age. I cross tabulated the BMI for age prior to measles illness or outbreak and measles illness. I then conducted bivariate analysis for wasting prior to measles illness or outbreak, age at exposure to measles, sex, CD4+ percentage prior to HAART initiation, and CD4+ percentage prior to measles illness or outbreak as independent variables and measles illness as the dependent variable to ascertain statistical significance

of any of the variables. I further conducted forward stepwise binomial logistic regression, adding one variable at a time to wasting in the model as described in the section on multivariate analysis above. I assessed the confounding or interaction effects on the relationship between wasting and measles illness at every step. The odds ratio of BMI for age and corresponding p value in the final model were used to ascertain whether wasting is a significant predictor of measles illness in HIV-infected children on HAART and to reject or not reject the null hypothesis.

Each nutrition status parameter was analyzed separately as they are measures of different types of malnutrition. Stunting is indicative of chronic under nutrition whereas wasting is a measure of acute disturbances in nutrition and growth. Underweight shows disharmony in both linear growth and body proportion (Shetty, n.d.). When all the three parameters for measuring under nutrition were included in the model, the standard error for the coefficient β for each of the parameters was less than one indicating that there is no collinearity between the three parameters.

CD4+ Count Prior to Measles Illness or Outbreak. To assess whether CD4+ count prior to measles was a significant predictor of the risk of measles in HIV-infected children on HAART, I compared the proportion of HIV-infected children on HAART with CD4+ percentage less than 25% prior to exposure to measles who developed measles with the proportion of the HIV-infected children on HAART with CD4+ percentage of 25% and above prior to measles outbreak that developed measles. Hence, cross tabulation was conducted for CD4+ count prior to measles (less than 25%; 25% and above) and measles illness. I conducted bivariate analysis for CD4+ percentage prior to

measles illness or outbreak, age, sex, CD4+ percentage prior to initiation of HAART, and duration on HAART as independent variables and measles illness as the dependent variable. Forward stepwise binomial logistic regression was then conducted including CD4+ percentage prior to measles illness or outbreak and the other variables (age, sex, CD4+ percentage prior to initiation of HAART, and duration on HAART) to ascertain statistical significance of any of them. I assessed for interaction and confounding by all the variables. Based on the result of the final model, inference was made on whether CD4+ prior to measles was a significant predictor of measles illness in HIV-infected children with HAART and the null hypothesis rejected or not rejected.

After assessment of the individual factors as possible predictors of the risk of measles individually, models were built for multivariate analysis (multiple logistic regression) to assess whether any of the factors (nutrition status, age at initiation of HAART, and CD4+ percentage prior to measles illness or outbreak) was significantly associated with measles illness in HIV-infected children on HAART when one or two of the other factors are controlled for. Different models were built using the different nutrition status parameters together with age at initiation of HAART and or CD4+ percentage prior to measles or measles outbreak to ascertain if any of them influenced risk of measles more than the others. More variables that could be confounders of the relationship between HIV infection (on HAART) and risk of measles like age, CD4+ percentage prior to HAART, and sex were added to the logistic regression models above. Forward stepwise binomial logistic regression was used as described in the section on

multivariate analysis to conduct a more comprehensive analysis of the significant risk

factors for measles among HIV-infected children on HAART.

Internal and External Validity

Threats to Validity of Design

Potential threats to validity of design in this study could include missing data due

to retrospective data collection nature of the study, and misclassification of exposure

(Mann, 2003; Song and Chung, 2010).   To minimize selection bias, study participants

were randomly selected from the list of all eligible exposed children extracted from the

HCD database and from a list of eligible unexposed children extracted from the OPD

database. To minimize further selection bias, all eligible exposed and unexposed children

identified from the HCD database and OPD database respectively were included in the

sampling frame. Even the children who could have died due to measles or any other

cause during or after measles outbreak were included in the sampling frame.

There was a potential threat of misclassification of HIV-uninfected (unexposed)

by the time of the measles outbreak if a positive HIV diagnosis was not recorded on the

OPD database or register and the child was included as an unexposed participant.

However, given the commitment at Nsambya hospital to get all HIV-infected children

into care at HCD early in life (Massavon et al., 2013), this was highly unlikely, though

possible. To assess the possibility of the misclassification, the names of selected children

from the OPD with measles were shared with the HCD data manager to conduct a cross

check in the HCD database, however none of the names were found in this database. HIV

infection was included in the diagnoses of several children in the OPD database and

register; such children were excluded from the sampling frame. To minimize missing and

inaccurate data, medical records were used to collect all key data, especially CD4+

count/percentage prior to initiation of HAART, date of HAART initiation, CD4+

count/percentage prior to measles illness, weight and height measurements prior to

measles illness or outbreak in HIV-infected children on HAART. Nevertheless, some

information was missed out on some cases as it had not been recorded. Where the

missing information was more than 50% of expected, for example immunization status of

un-exposed children, the observation was excluded from the analysis. While conducting

analysis using SPSS, the observations with missing data were excluded from analysis in

general.

Threats to Validity of Measurement

The study could have also faced threats of validity of measurement if the data

collection tool was not clear or with variables not defined clearly or useful in addressing

the study questions (content validity) (Frankfort-Nachmias and Nachmias, 2008). To

minimize threats to measurement validity, the tool was shared with one national expert in

measles and one national expert in HIV, as well as my dissertation committee for

assessment of content validity and no questions were indicated as unclear. After

enrollment, medical records of 10 randomly selected study participants (5 exposed and 5

unexposed children) were reviewed again on different days from the first review and data

collected in the second review compared with that collected in the first review to assess

validity of the tool and reliability of data being collected (Frankfort-Nachmias and

Nachmias, 2008). Data collected in the first review was 100% same as the data from the second review.

External Validity

The main threat for external validity is getting a non-representative sample such that results cannot be generalized to the population from which the sample was selected or beyond (Frankfort-Nachmias and Nachmias, 2008; Breslow, 2005). This could result from an inappropriate sample size and sampling procedure (Cresswell, 2009; Frankfort-Nachmias and Nachmias, 2008; Breslow, 2005). To enhance external validity, sample size estimation was carefully conducted and an adequate sample size based on estimated prevalence of the disease in exposed and unexposed children derived. The minimum sample size estimated a priori was obtained for all the analyses after excluding cases with missing variables, except in the analysis of the relationship between under nutrition on weight for age (underweight) and measles illness. In addition, random selection of exposed and unexposed children contributed to generating a representative sample. Given that Nsambya hospital attends to people from across Kampala and Wakiso from different socioeconomic strata, and the distribution of study participants in the different divisions and sub-counties in Kampala and Wakiso respectively, the sample generated was representative of the HIV-infected children on HAART and their HIV-uninfected peers in Kampala and Wakiso districts. General oversampling (not related to any characteristic) whereby sample size was increased by 10% prior to recruitment was conducted to increase possibility that an adequate sample would remain after dropping any cases that had key data missing on some variables, in the analysis. Furthermore, a higher than the

minimum sample size required was enrolled. However, given that the P_0 and P_1 attained in the study were significantly lower than estimated in the study design, even with the larger sample enrolled, the study power fell short of the desired 80%. Whereas the obtained sample was representative of the community from which it was drawn, it was not adequate to enable generalization of results beyond the study site.

<div align="center">

Ethical Issues

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Permission to Access Records

Permission was obtained from Nsambya hospital to access the HCD database, the clients' medical records, the OPD database and register, and where applicable the In-Patient medical records. As records were used for research purposes, personal identifiers were eliminated at extraction into the data collection tool. During data entry only study numbers were used. The data collected will be stored after initial analysis for at least 5 years to enable future reanalysis or review if necessary.

Confidentiality

Each participant was given a unique identification number and it was indicated on the data collection tool. When entering the data, the unique identifier was entered instead of the name to delink the data from the names of the participants. All data collected was kept confidential, with restricted access by only the principal investigator (PI) and assisting statistician. The hard copies of the completed data collection forms will be stored in a locked cabinet for at least 5 years, with access limited to the PI. There were no incidents of breach in confidentiality during the course of the study; hence, there was no need for corrective measures to ensure confidentiality.

Institutional Review and Approval

Institutional Review Board approval was obtained from Walden University

(Number 03-09-15-0275826) before data collection was initiated. In addition, scientific

and ethical approval from the institutional review boards (IRB) at Nsambya Hospital, The

AIDS Support Organization (TASO), and Uganda National Council of Science and

Technology were sought. The IRB at TASO is accredited by the Uganda National

Council of Science and Technology. Finally, approval was sought from the Uganda

President's Office Research Department, through the National Council of Science and

Technology.

Summary

In this chapter, I described the methodology used in this study. The purpose of

this study was to examine whether there is a difference between the risk of measles in

HIV-infected children on HAART and that of HIV-uninfected children of the same age

group in Uganda; to investigate the relationship between selected risk factors (nutritional

status, age at initiation of HAART, and low CD4+ count) and the risk of measles in HIV-

infected children on HAART; and to ascertain the age group where HIV-infected children

on HAART are most affected by measles, in order to determine the most appropriate

timing for revaccination, if necessary. A retrospective cohort study design was used and

the study was implemented at Nsambya Hospital in Kampala, Uganda. Study approval

was obtained from the IRB of Walden University, Nsambya hospital, TASO, and the

Uganda National Council of Science and Technology. Permission to access medical

records for research purposes was also obtained from Nsambya hospital. Data was

collected from medical records onto a developed data collection tool. Data was double

entered into Epi Info 7 data entry screens. Descriptive epidemiological analysis, bivariate,

and multivariate analysis methods were used, using SPSS version 21 software, to answer

the study questions. The results of the study are presented in Chapter 4 and the

conclusions and implications of the findings in Chapter 5.

Chapter 4: Results

Introduction

The main purpose of this study was to examine whether there was a difference between the risk of measles in HIV-infected children on HAART and that of HIV-uninfected children of the same age group in Uganda using a retrospective cohort study design. I also investigated the relationship between nutritional status (undernutrition), age at initiation of HAART, and low CD4+ count and the risk of measles for HIV-infected children on HAART. Lastly, I assessed the age group most affected by measles among HIV-infected children on HAART, to determine the appropriate timing for revaccination, if necessary. In this chapter, I will describe the findings of this quantitative study question by question. I will discuss the interpretation of the study results conclusions and recommendations for further research in Chapter 5.

Research Questions and Hypotheses

This study sought to answer the following research questions and test the following hypotheses:

Primary Research Question:

PQ 1. Is there a difference in the risk of measles among HIV-infected children on HAART compared to uninfected children of the same age group?

$H_0$1: There is no difference between the risk of measles among HIV-infected children on HAART and the risk of measles among HIV-uninfected children of the same age group.

H_a1: There is a difference between the risk of measles in HIV-infected children on HAART and the risk of measles among HIV-uninfected children of the same age group.

Secondary Research Questions:

RQ 1. What is the age group most affected by measles among the HIV-infected children on HAART?

RQ 2. Are age at initiation of HAART, low nutritional status (undernutrition), and low CD4+ count significant predictors of measles in HIV-infected children on HAART?

H_02: Age at initiation of HAART is not a significant predictor of measles in HIV-infected children on HAART.

H_a2: Age at initiation of HAART is a significant predictor of measles in HIV-infected children on HAART.

H_03: Nutritional status (undernutrition) is not a significant predictor of measles in HIV-infected children on HAART.

H_a3: Nutritional status (undernutrition) is a significant predictor of measles in HIV-infected children on HAART.

H_04: Low CD4+ count is not a significant predictor of measles in HIV-infected children on HAART.

H_a4: Low CD4+ count is a significant predictor of measles in HIV-infected children on HAART.

Study Findings

Description of the Sample

At Nsambya HCD, 625 HIV-infected children from Kampala, Wakiso, Mukono, and Luwero districts were enrolled into care; 344 had been initiated on HAART. By November 2011, there were 313 "active" children on HAART as 31 had died, transferred to other HAART centers, or were unavailable for follow up. Of the 313 children, 2 were from Mukono district and were excluded from the sampling frame. Thus, the required sample of exposed children (223) was randomly selected from the 311 "active" children who resided in Kampala or Wakiso.

A total of 3,620 children less than 15 years old from Kampala, Wakiso, Mukono, Luwero, and a few districts beyond the catchment area of the hospital attended Nsambya OPD for care. Of the 3,620 children, 1288 (35.5%) were between 2 to 15 years old. Eight of the children 2 to 15 years of age had been diagnosed with HIV and were removed from the sampling frame before the random selection of the unexposed children was conducted. Additionally, 80 children 2 to 15 years of age resided in districts other than Kampala and Wakiso and were removed from the sampling frame. Hence, the sample of unexposed children was randomly selected from the remaining list of 1200 children. A total of 446 children were included in the study sample; 223 were exposed and 223 were unexposed.

Descriptive Epidemiology

Age and Sex Distribution

The mean age of all the children in the study sample was 7.53 years. The age distribution of children in the total sample was slightly skewed to the left, with most of the children less than 10 years of age. The age distribution of the unexposed children was skewed to the left while that of the exposed was skewed to the right but closer to a normal distribution. The mean age of the unexposed was significantly lower than the mean age of the exposed children (5.6 versus 9.5 years; $p < 0.001$) (Figure 4). Fifty one percent (228 out of 446) of children in the study sample were females while 49% were males. Among the exposed, 49% and 51% were female and male children respectively. Of the unexposed, 53.4% and 46.6% were female and male children respectively.
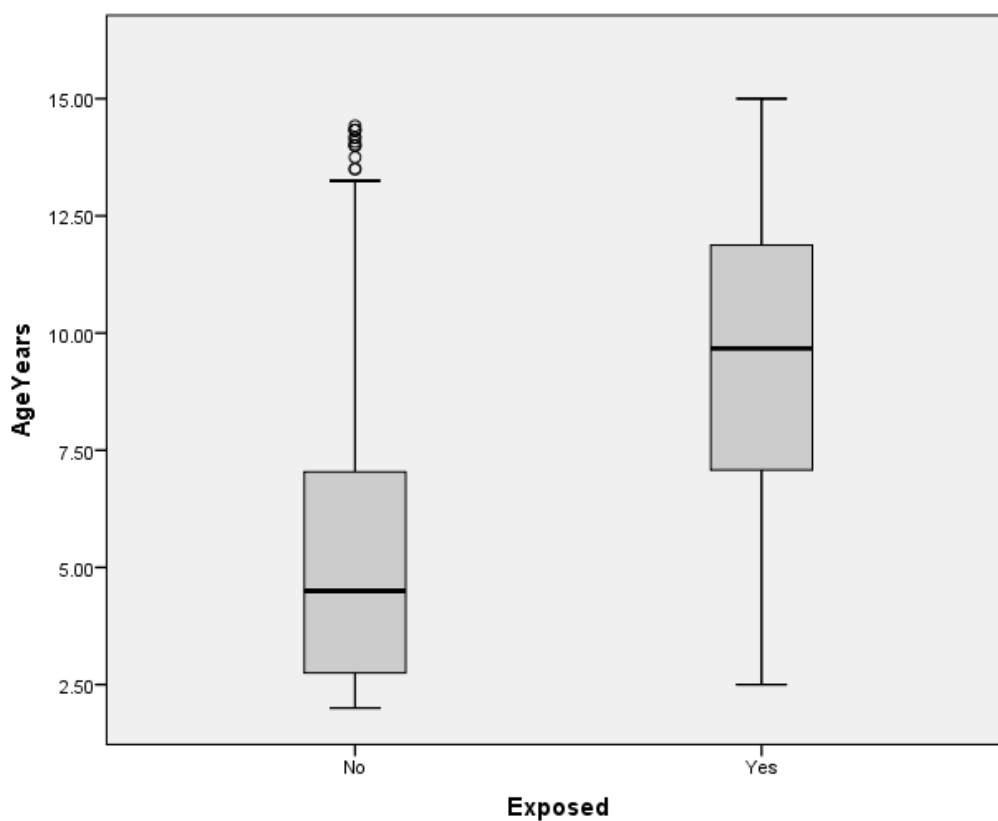
Figure 4. Mean and range of age of the exposed and unexposed children in the study sample

Place

Most of the study participants (323, or72.4%) resided in Kampala district. About

60% of the exposed and unexposed children residing in Kampala district were in

Makindye division where Nsambya HCD is located, while very few exposed (3, or2%)

and none of unexposed children resided in Central division that is mainly a

commercial/business area of Kampala. Exposed and unexposed children selected from

Wakiso came from 12 and 8 of the 17 sub-counties of Wakiso district respectively. Thus

the study sample was well distributed across the two districts.

Measles illness

Of the 223 exposed children in the study, 12 (5.4%) suffered from measles illness between November 2011 to June 2012, and 14 children suffered from measles before November 2011. When the aforementioned 14 children that were not susceptible to measles by November 2011 are excluded from the denominator, the proportion of exposed children that developed measles between November 2011 and June 2012 was 5.7% (12/209). Of the 223 exposed children, 119 (53.4%) had been vaccinated through routine immunization at 9 months. Of the 119 vaccinated children, 14 (11.8%) suffered from measles compared to 12 out of 104 (11.5%) that were not vaccinated or whose vaccination status was unknown. After excluding the vaccinated children who did not suffer from measles from the denominator as they were possibly not susceptible, the proportion of exposed children that contracted measles was 26/118 (22%). The 118 children in the denominator comprise of 104 that were not vaccinated or had unknown vaccination status and 14 that were vaccinated but suffered from measles, an indication of susceptibility due to either primary or secondary failure.

A total of 94 measles cases were recorded among all children less than 15 years old who had been seen in the OPD between November 2011 and June 2012. Forty eight (51.1%) of the measles cases were less than 2 years of age while 46 (48.9%) were between 2 and 15 years. Hence, 2.1% (48/2332) of children less than 2 years old and 3.6% (46/1288) of children 2 to 15 years of age that attended OPD between November 2011 and June 2012 suffered from measles.

Out of the selected 223 unexposed children, 14 (6.3%) suffered from measles between November 2011 and December 2012. Out of the 223 children, only 16 (7.2%) had records indicating that they were vaccinated; out of the 16, five (31.3%) contracted measles. When the remaining 11 vaccinated unexposed children were excluded from the denominator based on the assumption that they were not susceptible, the proportion of unexposed children who contracted measles was 14/212 (6.6%). The mean age of all study participants who suffered from measles in the time period of interest was 6.27 years; however, the mean age of the exposed children that suffered from measles during this time period was significantly higher than the mean age of the unexposed children that suffered from measles during the same period (8.02 versus 5.45; $p = 0.013$). The descriptive statistics of the study sample are summarized in Tables 3, 4 and 5.

Table 3

Summary of Descriptive Statistics of the Study Sample (n = 446)

Variable	Exposed (*n*=223)	Unexposed (*n*= 223)	*p* value
Age at time of measles outbreak			
Mean [range]	9.5 [2.5, 15]	5.6 [2, 14.42]	< 0.001
Median	9.67	4.5	
Sex distribution (%)			
Male	51.1	46.6	0.34
Female	48.9	53.4	
Vaccinated against measles (%)			
Yes	53.4	7.2	
No	6.7	1.3	
Not Sure	39.9	91.5	
Proportion with measles illness (%)	5.4	6.3	0.686
Age at measles illness (November 2011 to June 2012)	8.02 [5.67, 12.08]	5.45 [2, 12.17]	0.013

Table 4

Summary of Descriptive Statistics of the Exposed Sample (n=223)

Variable	Frequency (%)	Mean [range]	Median
Age at Measles		6.68 [1.67, 12.08]	6.83
Sex Distribution			
Male	51.1		
Female	48.9		
Measles vaccination			
Yes	53.4		
No	6.7		
Not Sure	39.9		
Age at HAART initiation		5.67 [0.33, 12.58]	5.67
Duration on HAART		3.85 [0.17, 12.09]	3.91
CD4+ count (cells/µl) at HAART initiation		481 [5, 2367]	388
CD4+ percentage at HAART initiation (%)		11.02 [0.2, 28]	11
CD4+ count prior to measles illness or outbreak		938 [125, 2620]	852
CD4+ percentage prior to measles illness or outbreak (%)		25.3 [2.92, 53]	27.4
Weight for Age			
Normal nutrition status	53.85		
Undernutrition (Underweight)	30.77		
Height for Age			
Normal nutrition status	50.00		
Undernutrition (Stunting)	42.31		
Weight for Height			
Normal nutrition status	23.08		
Undernutrition (Wasting)	3.85		

Variable	Frequency (%)	Mean [range]	Median
Body Mass Index for age			
Normal nutrition status	80.77		
Undernutrition (Wasting)	7.69		

Table 5

Summary of Descriptive Statistics of Exposed Children with Measles and Exposed Children without measles (n=223)

Variable	Children who suffered from measles	Children who did not suffer from measles	*p* value
Age in years	*n* = 26	*n* = 197	
Mean	8.4	9.7	0.03
Median	7.8	9.9	
Sex (%)	*n* = 26	*n* = 197	
Male	58	50	0.48
Female	42	50	
Measles Vaccination Status (%)	*n* = 26	*n* = 197	
Vaccinated	53.8%	53.3%	0.96
Age at HAART Initiation in years	*n* = 26	*n* = 197	
Mean	4.7	5.8	0.07
Median	4.25	5.83	
Duration on HAART in years	*n* = 26	*n* = 197	
Mean	3.8	3.9	0.8
Median	4.0	3.83	
CD4+ count at HAART initiation	*n* = 26	*n* = 196	
Mean [range]	645 [15, 1794]	459 [5, 2367]	0.05
Median	542	379	
CD4+ percentage at HAART initiation	*n* = 23	*n* = 174	
Mean [range]	10.5 [0.8, 23.9]	11.09 [0.2, 28]	0.68
Median	11	11	

Variable	Children who suffered from measles	Children who did not suffer from measles	*p* value
CD4+ percentage prior to measles illness or measles outbreak	*n* = 20	*n* = 135	
Mean	26 [3.6, 53]	25 [2.9, 52.1]	0.64
Median	31	37	
Height for Age	*n* = 23	*n* = 155	
Undernutrition (Stunting) (%)	48	39	
Normal Nutrition (%)	52	61	0.44
Weight for Age	*n* = 22	*n* = 73	
Undernutrition (Underweight) (%)	36	21	0.13
Normal Nutrition (%)	64	79	
Body Mass Index for Age	*n* = 24	*n* = 155	
Undernutrition (Wasting) (%)	8.3	24	0.11
Normal Nutrition (%)	91.7	76	

Primary Research Question

PQ 1. Is there a difference in the risk of measles among HIV-infected children on HAART compared to uninfected children of the same age group?

*H₀*1: There is no difference between the risk of measles among HIV-infected children on HAART and the risk of measles among HIV-uninfected children of the same age group.

H_a1: There is a difference between the risk of measles in HIV-infected children

on HAART and the risk of measles among HIV-uninfected children of the same

age group.

Findings

Overall, 5.83% of the study participants suffered from measles between

November 2011 and June 2012; including 5.4% among the exposed and 6.3% among the

unexposed. The unadjusted crude relative risk was 0.857 [95% confidence interval (*CI*):

0.406, 1.812; *p* value = 0.686] (Table 6). However, when 14 exposed children that had

already suffered from measles were excluded from the analysis, 5.7% (12/209) of

exposed study participants suffered from measles between November 2011 and June

2012. In the analysis of the measles risk difference between the exposed and unexposed

children, the proportion of exposed that suffered from measles (5.4%) was used without

any exclusions.

Table 6

Proportion of Measles among Exposed Children Compared to Unexposed Children

	Measles	No Measles	Total	Risk Ratio	95% *CI*	Chi Square (X^2)	*p* value
Exposed	12	211	223				
Percent	**5.4**	94.6	100				
Unexposed	14	209	223				
Percent	**6.3**	93.7	100.				
Total	26	420	446				
Percent	5.8	94.2	100.	0.857	[0.406, 1.812]	0.163	0.686

The risk difference (*δ*) was 0.009 (95% *CI*: -0.001, 0.19). Given that the

confidence interval includes zero, the risk difference is not statistically significant.

Cohen's *h* was 0.364 indicating a small to medium effect size. Bivariate analysis was

conducted for age at time of measles outbreak and sex; odds ratio for age was 0.91 with *p*

value of 0.084 while for sex odds ratio was 0.95 and *p* value was 0.91 (Table 7). The risk

ratio for sex computed using a 2 by 2 contingency table was 1.046 (95% *CI*: 0.496,

2.205) with a *p* value of 0.91. As age was used as continuous variable, a contingency

table could not be used to assess the risk ratio.

Age at time of measles outbreak was a found to be a confounder of the

relationship between exposed status and measles illness (caused a 65% change in odds

ratio) whereas sex was not (0% change in odds ratio). Thus, sex was not included in the

multivariate model due to a very high *p* value and not being a confounder. The final

model thus had exposed status and age in years at time of the measles outbreak. The

adjusted odds ratio was 1.41 [95% *CI*: 0.533, 3.72; *p* = 0.49] (Table 7). Vaccination

status could not be adjusted for adequately due to lack of data on vaccination status for

most of the unexposed children. The model fitness and variance are shown in Table 8

below. Thus, although the exposed children had a 41% higher risk of measles than the

unexposed, this risk difference was not statistically significant. This is also indicated by

the confidence interval of the risk difference including zero. However, with a small to

medium effect size, the risk difference may be of clinical importance. The null hypothesis

thus could not be rejected based on these results.

Table 7

Bivariate and Multivariate Analysis for Variables in Age and Sex Distribution the

Primary Research Question

Variable	Crude odds ratio	*p* value	95% *CI*	Adjusted odds ratio	*p* value	95% *CI*
Age	0.91	0.084	[0.81, 1.01]	0.88	0.075	[0.77, 1.01]
Sex (Female/Male)	0.95	0.91	[0.43, 2.11]			
Exposed (Yes/No)	0.85	0.69	[0.38, 1.88]	1.41	0.49	[0.53, 3.72]

Table 8

Model Variance and Goodness of Fit for the Primary Research Question

Step	Nagelkerke pseudo R^2	Hosmer and Lemeshow Test		
		Chi square	Degrees of freedom	*p* value
Step 1 (Exposed Yes/No)	0.001	< 0.001	0	-
Step 2 (Exposed, age in years)	0.023	9.42	8	0.31
Step 3 (Exposed, age in years, sex (Female/Male)	0.023	6.60	8	0.58
Step 4 – Final Model (Exposed, age in years)	0.023	9.42	8	0.31

Secondary Research Questions

Secondary RQ 1: What is the age group most affected by measles among the HIV-infected children on HAART?

Most Affected Age Group among HIV-infected Children on HAART

Twenty-six study participants had suffered from measles while on HAART prior to and during the outbreak period (November 2011 to June 2012). Age at measles illness was available for all except one of the children on HAART. The age range of the exposed children that suffered from measles was 1.67 to 12.08 years. The most affected age group by measles among the HIV-infected children on HAART was the 5 to 9 years old children, followed by the 2 to 4 year old children (Figure 5).
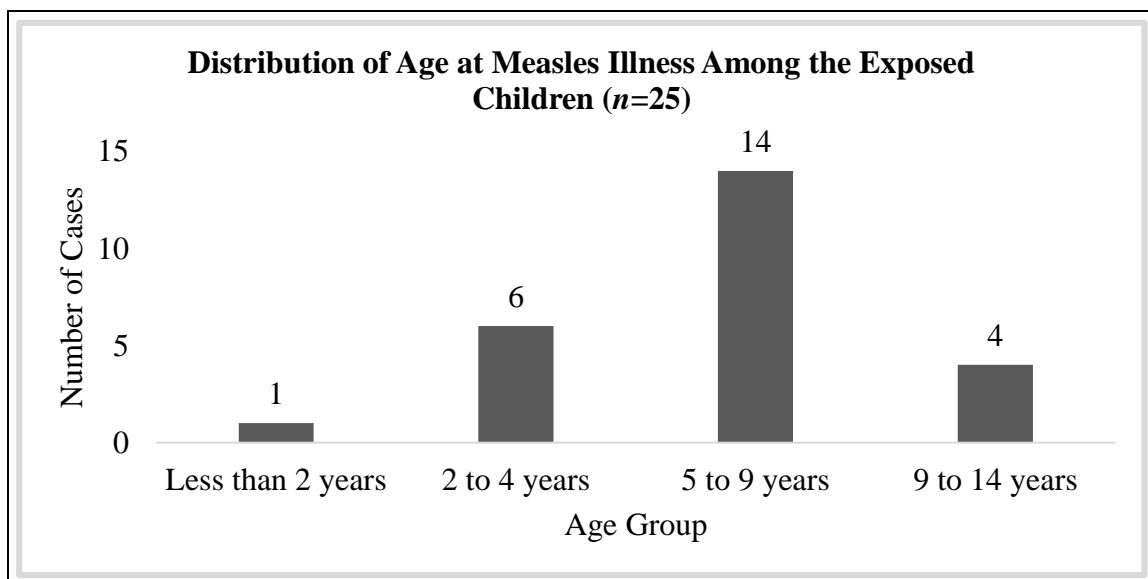
Figure 5. Age Distribution of HIV-infected Children on HAART that Suffered from Measles

Secondary RQ 2: Are age at initiation of HAART, low nutritional status (stunting, wasting, and underweight), and low CD4+ count prior to measles illness or

outbreak (CD4+ percentage of less than 25%) significant predictors of measles in HIV-infected children on HAART?

H$_0$2: Age at initiation of HAART is not a significant predictor of measles in HIV-infected children on HAART.

H$_a$2: Age at initiation of HAART is a significant predictor of measles in HIV-infected children on HAART.

H$_0$3: Nutritional status (undernutrition) is not a significant predictor of measles in HIV-infected children on HAART.

H$_a$3: Nutritional status (undernutrition) is a significant predictor of measles in HIV-infected children on HAART.

H$_0$4: Low CD4+ count is not a significant predictor of measles in HIV-infected children on HAART.

H$_a$4: Low CD4+ count is a significant predictor of measles in HIV-infected children on HAART.

Age at Initiation of HAART

The mean age at HAART initiation for exposed children that suffered from measles was 4.67 years (range from 0.67 years to 12.58 and median of 4.25) while that for exposed children that did not suffer from measles was 5.8 years (range from 0.33 to 12.42 years and median of 5.83). Only 10 (4.5%) of the exposed children were initiated on HAART at age below 9 months (0.75 years). Only one out of the 10 children (10%) who started HAART at age below 9 months suffered from measles while 25/213 (12%) of the children who started HAART at age 9 months and above developed measles. The

odds ratio was 0.836 [95% *CI*: 0.102, 6.867] and the *p* value was 0.867 (Table 9). The

mean duration on HAART for the exposed children who suffered from measles was 3.77

years (range from 0.25 to 6.08 years and median of 4 years) while that for the exposed

children that did not suffer from measles was 3.86 years (range from 0.17 to 12.09 years

and median of 3.83 years). Thirty eight out of 223 (17%) children had been on HAART

for less than 2 years by the beginning of the measles outbreak in November 2011. Sixteen

percent (6/38) of the children who had been on HAART for less than 2 years suffered

from measles compared to 11% (20/185) of the children that had been on HAART for 2

and above years, giving an odds ratio of 1.547 [95% *CI*: 0.576, 4.154] with a *p* value of

0.385 (Table 10).

Table 9

Age at Initiation of HAART and Measles in HIV-infected Children on HAART

	Measles	No measles	Total	Odds ratio	95% *CI*	*p* value
Less than 9 months	1	9	10			
Percent	10	90	100			
9 months and above	25	188	213			
Percent	12	88	100			
Total	26	197	223	0.836	[0.102, 6.867]	0.867

Table 10

Duration on HAART and Measles in HIV-infected Children on HAART

	Measles	No measles	Total	Odds ratio	95% *CI*	Chi Square	*p* value
Less than 2 years	6	32	38				
Percent	16	84	100				
2 years and above	20	165	185				
Percent	11	89	100				
Total	26	197	223	1.547	[0.576, 4.154]	0.755	0.385

Binomial logistic regression was conducted starting with age in years that had the lowest p-value in bivariate logistic regression. Age in years at time of measles outbreak was found to be a confounder of the relationship between age at initiation of HAART and measles and was thus left in the model. The CD4+ percentage prior to initiation of HAART was added to the model, also assessed and found to be a confounder, and left in the model. Sex and duration on HAART were also added one after the other but were not found to be confounders and were excluded from the model. Hence the final model included age at initiation of HAART, age at time of measles outbreak (years), and CD4+ percentage prior to initiation of HAART (Table 11). There was no collinearity between any of the independent variables included in the final model. The adjusted odds ratio for age at initiation of HAART was 0.49 (95% *CI*: 0.54, 4.44) with *p* value of 0.53. The models' fitness and variance are indicated as shown by Hosmer and Lemeshow Test and

Nagelkerke PseudoR^2 respectively, are shown in Table 12. Hence, there was no sufficient

evidence to reject the null hypothesis.

Table 11

Bivariate and Multivariate Analysis for Variables in Secondary Research Question 2.1

Variable	Crude odds ratio	95% *CI*	p value	Adjusted odds ratio	95% *CI*	*p* value
Age at HAART initiation (Less than 9 months/9 months and above)	0.84	[0.76, 1.01]	0.87	0.49	[0.54, 4.44]	0.53
CD4+ percent prior to HAART	0.98	[0.92, 1.06]	0.67	0.98	[0.91, 1.05]	0.51
Age in Years	0.88	[0.77, 1.00]	0.058	0.88	[0.75, 1.02]	0.095
Sex (Female/Male)	0.74	[0.32, 1.69]	0.48			
Duration on HAART	0.97	[0.78, 1.22]	0.82			

Table 12

Model Variance and Goodness of Fit

Step	Nagelkerke PseudoR^2 (%)	Hosmer and Lemeshow Test		
		Chi square	Degrees of freedom	*p* Value
Step 1 (Age at HAART Initiation (Less than 9months/9 months and above)	< 0.1	< 0.001	0	-
Step 2 (Age at HAART initiation, age in years)	3.7	11.09	8	0.20
Step 3 (Age at HAART initiation, age in years, CD4+ percent prior to HAART)	3	11.15	8	0.19
Step 4 (Age at HAART initiation, age in years, CD4+ percent prior to HAART, sex)	3.7	5.81	8	0.67
Step 5 (Age at HAART initiation, age in years, CD4+ percent prior to HAART, sex, duration on HAART)	3.7	7.06	8	0.53
Step 6 – Final Model (Age at HAART initiation (less than 9months/9 months and above), age in Years, CD4+ percent prior to HAART)	3	11.15	8	0.19

Low Nutrition Status

Stunting. In this study, 40.5% of the exposed participants were stunted. Of the 26 exposed children that suffered from measles, 23 had a z-score for height for age; 11 of the 23 children (48%) were stunted. Fifteen percent (11/72) of stunted children suffered from measles compared to 11% (12/106) of children that were not stunted. When measles occurrence among the stunted exposed children was compared to that in exposed and not stunted children, the odds ratio was 1.413 [0.586, 3.403], with a *p* value of 0.44 (Table 13). This result indicates that whereas the odds of suffering from measles for stunted exposed children was 41.3% higher than for those not stunted, the difference was not statistically significant.

Table 13

Height for Age and Measles in HIV-infected Children on HAART

	Measles	No measles	Total	Odds ratio	95% *CI*	Chi square	*p* value
Undernutrition (Stunted)	11	61	72				
Percent	15	85	100				
Normal Nutrition	12	94	106				
Percent	11	89	100				
Total	23	155	178	1.413	[0.586, 3.403]	0.593	0.441

Binomial logistic regression was done starting with age in years that had the lowest *p* value. From the model, age was a significant predictor of measles with a *p* value

of 0.05 and was retained in the model together with stunted. I assessed for interaction between age in years and stunting using the chunk test and found that age was not interacting with stunting. In the next step, CD4+ percentage prior to HAART was included in the model, assessed for confounding at 10% cut off, and found to be a confounder of the relationship between stunting and measles. CD4+ percentage prior to HAART was thus also retained in the model. In the next step, sex was included in the model, assessed for confounding and found not to be a confounder of the relationship between stunting and measles, and was excluded from the model. In the next step, I included CD4+ percentage prior to measles or measles outbreak, assessed for confounding, found this to be a confounder of the relationship between stunting and measles, and thus left it in the model.

Hence the final model included stunting, age in years at time of measles outbreak, CD4+ percentage prior to HAART, CD4+ percentage prior to measles or measles outbreak (see Table 15). There was no collinearity between any of the independent variables included in the final model. The adjusted odds ratio of stunting in the final model was 4.14 with a p value of 0.02 (Table 14). The models' fitness and variance as shown by Hosmer and Lemeshow Test and Nagelkerke PseudoR^2 respectively, are indicated in Table 15; the Nagelkerke Pseudo R^2 indicates that only 14% of the variance is explained by the final model. From these findings, stunting is a significant predictor of measles in HIV-infected children on HAART. The null hypothesis indicating that undernutrition (stunting) is not a predictor of measles in HIV-infected children on HAART was thus rejected.

Table 14

Bivariate and Multivariate Analysis for Variables in Secondary Research Question 2.2.1

Variable	Crude odds ratio	95% *CI*	*p* value	Adjusted odds ratio	95% *CI*	*p* value
Stunting	1.41	[0.586, 3.40]	0.44	4.14	[1.25, 13.71]	0.02
Age in years	0.88	[0.77, 1.00]	0.058	0.80	[0.65, 0.99]	0.04
Sex (Female/Male)	0.74	[0.32, 1.69]	0.48			
CD4+ percent prior to HAART	0.98	[0.92, 1.06]	0.67	0.94	[0.86, 1.04]	0.21
CD4 percent prior to measles illness or outbreak	1.01	[0.94, 1.042]	0.68	0.99	[0.95, 1.04]	0.73

Table 15

Model Variance and Goodness of Fit for Secondary Research Question 2.2.1

Step	Nagelkerke PseudoR^2 (%)	Hosmer and Lemeshow Test		
		Chi square	Degrees of freedom	*p* value
Step 1 (Stunted)	0.6	0.000	0	-
Step 2 (Stunted, age in years)	9.2	3.92	8	0.86
Step 3 (Stunted, age in years, CD4+ percent prior to HAART)	11.8	6.4	8	0.60
Step 4 (Stunted, age in years, CD4+ percent prior to HAART, sex)	12.6	6.31	8	0.61
Step 5 (Stunted, age in years, CD4+ percent prior to HAART, CD4+ percent prior to measles /measles outbreak)	14.2	8.3	8	0.41
Step 6 - Final Model (Stunted, age in years, CD4+ percent prior to HAART, CD4+ percent prior to measles /measles outbreak)	14.2	8.3	8	0.41

Underweight. Weight for age z-score was available for 95 of the exposed

children including 22 who suffered from measles. Among the 95 exposed children with

weight for age z-score in this study, 23 (24%) were underweight. Among the exposed

children that suffered from measles 8/22 (36.4%) were underweight. Thirty five percent

(8/23) of the underweight children suffered from measles compared to 19% (14/72) of the

exposed children that were not underweight, giving a crude odds ratio of 2.210 [0.783,

6.237] with a *p* value of 0.13 (Table 16). Thus the odds of suffering from measles for

HIV-infected children on HAART that were underweight was about 2 times that of HIV-

infected children on HAART that are not underweight though this was not statistically

significant.

Table 16

Weight for Age and Measles among HIV-infected Children on HAART

	Measles	No measles	Total	Odds ratio	95% *CI*	Chi square	*p* value
Undernutrition (Underweight)	8	15	23				
Percent	35	65	100				
Normal Nutrition	14	58	72				
Percent	19	81	100				
Total	22	73	95	2.21	[0.783, 6.237]	2.28	0.13

Simultaneous binomial logistic regression was conducted to assess the effect of

age, sex, CD4+ percentage prior to HAART, and CD4+ percentage prior to measles or

measles outbreak on the relationship between underweight and measles in HIV-infected

children on HAART. I also assessed for confounding effect of each variable included in the model using 10% change in odds ratio as the cut off. All the above four variables were found to be confounders of the relationship between underweight and measles and were all included in the model. Hence the model included underweight, age in years, sex, CD4+ percentage prior to HAART, CD4+ percentage prior to measles or measles outbreak (see Table 18). There was no collinearity between any of the independent variables included in the final model. The adjusted odds ratio for underweight was 2.69 with a p value of 0.13 (Table 17). The models' fitness and variance as shown by Hosmer and Lemeshow Test and Nagelkerke Pseudo R^2 respectively, are indicated in Table 18; the Nagelkerke Pseudo R^2 indicates that only 18% of the variance is explained by the final model. Hence from these findings, the odds of measles in underweight HIV-infected children on HAART was 2.7 times that of HIV-infected children on HAART with normal weight. However, underweight was not a significant predictor of measles in HIV-infected children on HAART. The null hypothesis indicating that undernutrition (underweight) is not a predictor of measles in HIV-infected children on HAART thus could not be rejected.

Table 17

Bivariate and Multivariate Analysis for Variables in Secondary Research Question 2.2.2

Variable	Crude odds ratio	95% *CI*	*p* value	Adjusted odds ratio	95% *CI*	*p* value
Underweight	2.21	[0.78, 6.24]	0.13	2.69	[0.74, 9.72]	0.13
Age in Years	0.88	[0.77, 1.00]	0.06	1.20	[0.91, 1.57]	0.20
Sex (Female/Male)	0.74	[0.32, 1.69]	0.48	0.63	[0.18, 2.28]	0.49
CD4+ percent prior to HAART	0.98	[0.92, 1.06]	0.67	0.92	[0.82, 1.02]	0.12
CD4 percent prior to measles illness or outbreak	1.007	[0.91, 1.04]	0.68	1.01	[0.96, 1.05]	0.84

Table 18

Model Variance and Goodness of Fit for Secondary Research Question 2.2.2

Step	Nagelkerke PseudoR^2 (%)	Hosmer and Lemeshow Test		
		Chi square	Degrees of freedom	*p* value
Final model (Underweight, age in years, Sex, CD4+ percent prior to HAART, CD4+ percent prior to measles illness or outbreak)	18.3	6.38	8	0.61

Wasting. Body Mass Index (BMI) for age was available for 179 exposed children including 24 of the children who suffered from measles. Among the exposed children in this study 39/179 (22%) were wasted; among the exposed children with measles, 2/24 (8%) were wasted. Only two (5%) of the exposed children who were wasted suffered from measles compared to 16% of exposed children that were not wasted giving an odds ratio of 0.29 [95% *CI*: 0.065, 1.291] with a *p* value of 0.111 (Table 19).

Table 19

Wasting and Measles among HIV-infected Children on HAART

	Measles	No measles	Total	Odds ratio	95% *CI*	*p* value (Fisher's exact)
Undernutrition (Wasting)	2	37	39			
Percent	5	95	100			
Normal Nutrition	22	118	140			
Percent	16	84	100			
Total	24	155	179	0.290	[0.065, 1.291]	0.111

Binomial logistic regression was conducted to assess the effect of age, sex, CD4+ percentage prior to HAART, and CD4+ percentage prior to measles or measles outbreak on the relationship between wasting and measles in HIV-infected children on HAART. I also assessed for confounding effect of each variable added into the model. All the above variables except sex were found to be confounders of the relationship between wasting and measles and were thus retained in the final model. Hence the final model included wasting, age in years, CD4+ percentage prior to HAART, CD4+ percentage prior to

measles or measles outbreak (see Table 21). There was no collinearity between any of the

independent variables included in the final model. The adjusted odds ratio for wasting in

the final model was 0.5 with a *p* value of 0.39 (Table 20). The models' fitness and

variance as shown by Hosmer and Lemeshow Test and Nagelkerke Pseudo R^2

respectively, are indicated in Table 21; the Nagelkerke Pseudo R^2 indicates that only 5%

of the variance is explained by the final model. Hence, from these findings, wasting was

not a significant predictor of measles in HIV-infected children on HAART. The null

hypothesis indicating that undernutrition (wasting) is not a predictor of measles in HIV-

infected children on HAART thus could not be rejected.

Table 20

Bivariate and Multivariate Analysis for Variables in Secondary Research Question 2.2.3

Variable	Crude odds ratio	95% *CI*	*p* value	Adjusted odds ratio	95% *CI*	*p* value
Wasting	0.29	[0.07, 1.29]	0.10	0.50	[0.10, 2.42]	0.39
Age in Years	0.88	[0.77, 1.00]	0.058	0.91	[0.76, 1.09]	0.31
Sex (Female/Male)	0.74	[0.32, 1.69]	0.48			
CD4+ percent prior to HAART	0.98	[0.92, 1.06]	0.67	0.94	[0.86, 1.03]	0.21
CD4 percent prior to measles illness or outbreak	1.007	[0.91, 1.04]	0.68	0.99	[0.96, 1.04]	0.77

Table 21

Model Variance and Goodness of Fit for Secondary Research Question 2.2.3

Step	Nagelkerke PseudoR^2 (%)	Hosmer and Lemeshow Test		
		Chi square	Degrees of freedom	*p* value
Step 1 (Wasting)	3.6	0.000	0	-
Step 2 (Wasting, age in years)	7.2	13.31	8	0.10
Step 3 (Wasting, age in years, CD4+ percent prior to HAART)	6.7	10.12	8	0.26
Step 4 (Wasting, age in years, CD4+ percent prior to HAART, sex)	7.9	5.15	8	0.74
Step 5 (Wasting, age in years, CD4+ percent prior to HAART, CD4+ percent prior to measles illness or outbreak)	5.4	11.48	8	0.18
Step 6 – Final model (Wasting, age in years, CD4+ percent prior to HAART, CD4+ percent prior to measles illness or outbreak)	5.4	11.48	8	0.18

CD4+ Count Prior to Measles

The data on CD4+ percentage prior to measles illness was available for 21 out of the 26 exposed children that suffered from measles. Data on CD4+ percentage prior to the measles outbreak was available for 135 children out of the 197 exposed children that did not suffer from measles. The mean CD4+ percent prior to measles among the exposed children that suffered from measles was 26.4% (ranging from 3.6% to 53%) and the median was 30.8%, while the mean CD4+ percent for exposed children that did not suffer from measles was 25.1% (ranging from 2.92% to 52.3%) and the median was 27.1%. The mean CD4+ percentage prior to measles illness among the exposed children that suffered from measles was not significantly lower than the mean CD4+ percentage prior to the measles outbreak for exposed children that did not suffer from measles (26.4% versus 25.1%) with a *p* value of 0.64. When measles occurrence among the exposed children with CD4+ percent less than 25% was compared to that among exposed children with CD4+ percent of 25% and above, the unadjusted odds ratio was 1.099 [*CI*: 0.434, 2.781] with a *p* value of 0.842 (Table 22).

Before the November measles outbreak, Nsambya HCD mainly used CD4+ percentage of 15% as the threshold for initiation of HAART. Using this as the cut off in the analysis of the relationship between CD4+ percentage prior to measles outbreak or measles illness and measles illness showed that the odds of suffering from measles for exposed children with CD4+ percent less than 15% (severe immunosuppression) compared to exposed children with CD4+ percent of 15% and above was slightly higher at 1.208 [95% *CI*: 0.300, 2.278] with a *p* value of 0.714 (Table 23).

Table 22

CD4+ prior to Measles Illness or Outbreak in Exposed Children

	Measles	No measles	Total	Odds ratio	95% *CI*	Chi square	*p* value
CD4+ less than 25%	9	61	70				
Percent	13	87	100				
CD4+ 25% and above	12	74	86				
Percent	14	86	100				
Total	21	135	156	0.91	[0.360, 2.302]	0.040	0.842

Table 23

CD4+ prior to Measles Illness and Outbreak in Exposed Children using Severe Immune Compromised Cut Off

	Measles	No measles	Total	Odds ratio	95% *CI*	Chi square	*p* value
CD4+ less than 15%	6	44	50				
Percent	12	88	100				
CD4+ 15% and above	15	91	106				
Percent	14	86	100				
Total	21	135	156	1.208	[0.30, 2.278]	0.134	0.714

Binomial logistic regression was conducted to assess the effect of age, sex, CD4+ percentage prior to HAART, on the relationship between CD4+ percentage prior to measles or measles outbreak and measles in HIV-infected children on HAART. Confounding effect of each variable added into the model was also assessed. Only CD4+ percentage prior to HAART was found to be a confounder of the relationship between CD4+ percentage prior to measles or measles outbreak and measles and was thus retained in the final model.

Hence the final model included CD4+ percentage prior to HAART and CD4+ percentage prior to measles or measles outbreak (see Table 25). There was no collinearity between any of the independent variables included in the final model. The adjusted odds ratio for CD4+ percentage prior to measles or measles outbreak in the final model was 1.16 with a *p* value of 0.78 (Table 24). The models' fitness and variance as shown by Hosmer and Lemeshow Test and Nagelkerke Pseudo R^2 respectively, are indicated in Table 25; the Nagelkerke Pseudo R^2 indicates that only 2% of the variance is explained by the final model. Hence, from these findings, CD4+ percentage prior to measles was not a significant predictor of measles in HIV-infected children on HAART. The null hypothesis indicating that CD4+ percentage prior to exposure to measles is not a predictor of measles in HIV-infected children on HAART thus could not be rejected.

Table 24

Bivariate and Multivariate Analysis for Variables in Secondary Research Question 2.3

Variable	Crude odds ratio	95% *CI*	*p* value	Adjusted odds ratio	95% *CI*	*p* value
CD4 percent prior to measles illness or outbreak (less than 25%/25% and above)	0.91	[0.36, 2.30]	0.84	1.16	[0.42, 3.16]	0.78
CD4+ percent prior to HAART	0.98	[0.92, 1.06]	0.67	0.95	[0.87, 1.04]	0.25
Age in years	0.88	[0.77, 1.00]	0.058			
Sex (Female/Male)	0.74	[0.32, 1.69]	0.48			

Table 25

Model Variance and Goodness of Fit for Secondary Research Question 2.3

Step	Nagelkerke PseudoR^2 (%)	Hosmer and Lemeshow Test		
		Chi square	Degrees of freedom	*p* value
Step 1 (CD4 percent prior to measles illness or outbreak (less than 25%/25% and above)	0	0.000	0	-
Step 2 (CD4 percent prior to measles illness or outbreak (less than 25%/25% and above), CD4+ percent prior to HAART)	2.1	3.14	8	0.93
Step 3 (CD4 percent prior to measles illness or outbreak (less than 25%/25% and above), CD4+ percent prior to HAART, age in years)	3.5	6.7	8	0.57
Step 4 – Final model (CD4 percent prior to measles illness or outbreak (less than 25%/25% and above), CD4+ percent prior to HAART)	2.1	3.14	8	0.93

After assessment of age at initiation of HAART, low nutrition status including stunting, underweight and wasting, and CD4+ prior to measles outbreak or illness as possible predictors of the risk of measles individually, binomial logistic regression was conducted including the age at initiation of HAART, CD4+ percentage prior to measles, and the different parameters of nutrition status. There was no collinearity between the three undernutrition parameters (stunting, wasting, and underweight). The model with stunting among the three nutrition status parameters turned out the best fitting with almost significant odds ratio for stunting and age at initiation of HAART (Table 26). When confounders age in years at time of measles outbreak and CD4+ percentage prior to HAART were included in the above model, Nagelkerke pseudoR^2 was 14.7% and stunting emerged the only statistically significant predictor of measles in HIV-infected children on HAART, with an odds ratio of 4.09 and a p value of 0.02 (Table 27).

Table 26

Age at Initiation of HAART, CD4+ prior to Measles, Stunting, and Measles in HIV-infected Children on HAART

Variable	Odds ratio	95% *CI*	*p* value
Stunting	2.51	[0.92, 6.88]	0.07
Age at initiation of HAART	0.86	[0.72, 1.03]	0.096
CD4+ percentage prior to measles	1.04	[0.39, 2.78]	0.93

Table 27

Age at Initiation of HAART, CD4+ prior to Measles, Stunting, Age, CD4+ prior to HAART and Measles in HIV-infected Children on HAART

Variable	Odds ratio	95% *CI*	*p* value
Stunting	4.09	[1.24, 13.43]	0.02
CD4+ percentage prior to HAART	0.98	[0.86, 1.04]	0.22
Age in years	0.88	[0.62, 1.27]	0.50
Age at initiation of HAART	0.89	[0.63, 1.27]	0.52
CD4+ percentage prior to measles	1.28	[0.43, 3.88]	0.66

Summary

The main study finding shows that there was no significant difference in the risk of measles among HIV-infected children on HAART compared to HIV-uninfected children in the same age group. After logistic regression, the relative risk increased from 0.86 (unadjusted) to 1.41 (adjusted) indicating that HIV-infected children on HAART had a 41% higher risk of measles that the HIV-uninfected children of the same age group. However this risk difference was still not statistically significant (*p* value of 0.49). Age at time of measles outbreak was found to be a confounder of the relationship between exposed status and measles. Although, the null hypothesis for the primary research question could not be rejected, the small to medium effect size indicates that the risk difference may have some clinical importance. The power of the study was low due to the small numbers of measles cases that were recorded among the selected exposed and

unexposed children, thus results of the study may not be conclusive on the measles risk difference between HIV-infected children on HAART and HIV-uninfected children.

Secondary RQ 1 was about the age group most affected by measles among the HIV-infected children on HAART. The most affected by measles among the HIV-infected children on HAART was the 5 to 9 years age group, followed by the 2 to 4 years age group. In secondary RQ 2, I investigated whether age at initiation of HAART was a significant predictor of measles in HIV-infected children on HAART. The results showed that there was no statistically significant difference in age at initiation of HAART between the HIV-infected children on HAART that suffered from measles and HIV-infected children on HAART that did not suffer from measles. Ten percent of children that initiated HAART at age less than 9 months suffered from measles compared to 12% of children who initiated HAART at 9 months and above. The average duration on HAART was 3.8 years and 3.9 years for the children that suffered from measles and those that did not suffer from measles respectively. The odds of suffering from measles for children who were initiated on HAART before 9 months was half that of children who were initiated on HAART at 9 months and above, although the difference was not statistically significant. Hence, there was no sufficient evidence to reject the null hypothesis.

Secondary RQ 2 also examined whether nutrition status (stunting) prior to measles outbreak was a significant predictor of measles in HIV-infected children on HAART. Fifteen percent (15%) of exposed stunted children suffered from measles compared to 11% among exposed but not stunted children (OR 1.4; $p = 0.44$). When

binomial logistic regression was conducted, the adjusted odds ratio for stunting increased to 4.14 ($p = 0.02$) showing that stunting was a significant predictor of measles in HIV-infected children on HAART.

Regarding weight for age, underweight was not a significant predictor of measles in HIV-infected children on HAART. The unadjusted odds ratio for measles among underweight HIV-infected children on HAART compared to HIV-infected children on HAART that were not underweight was 2.210 ($p = 0.13$). On binomial logistic regression, the adjusted odds of measles in underweight HIV-infected children on HAART increased to 2.7 but this result was not statistically significant.

Pertaining to weight for height, Body Mass Index for age was the parameter used to assess under nutrition (wasting). On logistic regression, the adjusted odds ratio for wasting, which was 0.5 ($p = 0.39$) indicated that wasting was not a significant predictor of measles in HIV-infected children on HAART. In summary, based on the findings pertaining to low nutritional status, stunting was a significant predictor of measles in HIV-infected children on HAART while underweight and wasting were not.

In secondary RQ 2 I further investigated whether CD4+ count prior to measles or measles outbreak was a significant predictor for measles in HIV-infected children on HAART. The unadjusted odds ratio for measles among HIV-infected children on HAART with CD4+ percent prior to measles of less than 25% (immune suppressed) compared to those with CD4+ percentage of 25% and above (not immune suppressed) was 1.099 ($p = 0.84$). The adjusted odds ratio for CD4+ percentage prior to measles or measles outbreak in the final logistic regression model was 1.16 with a p-value of 0.78.

Hence, from these findings, CD4+ percentage prior to measles was not a significant predictor of measles in HIV-infected children on HAART. There was thus insufficient evidence to reject the null hypothesis.

   After assessment of the several factors as possible predictors of the risk of measles individually, multivariate analysis was conducted including the age at initiation of HAART, CD4+ percentage prior to measles, and the different parameters of nutrition status. The model with stunting among the three nutrition status parameters turned out the best with almost significant odds ratio for stunting and age at initiation of HAART. When confounders age at time of measles outbreak and CD4+ percentage prior to HAART were included in the model, stunting emerged the only statistically significant predictor of measles in HIV-infected children on HAART (OR = 4.09; p = 0.02). The implications for these findings of the study are discussed in Chapter 5.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

The main purpose of this study was to examine whether there was a difference between the risk of measles in HIV-infected children on HAART and that of HIV-uninfected children of the same age group in Uganda using a retrospective cohort study design. I also investigated the relationship between age at initiation of HAART, under nutrition, and low CD4+ count and the risk of measles in HIV-infected children on HAART. Lastly, I assessed the age group most affected by measles among HIV-infected children on HAART to determine the appropriate timing for revaccination, if necessary. In this chapter, I interpret and discuss the findings of the study, draw conclusions from the study based on the findings, compare these findings to past literature and the conceptual framework. Finally, I highlight recommendations from the study and the implications for positive social change.

Summary of Results

Results of this study showed that there was no statistically significant difference between the risk of measles between HIV-infected children on HAART and HIV-uninfected children of the same age group. However, the results showed a small to medium effect size (0.364) that may be of some clinical importance. Among the HIV-infected children on HAART, the age group most affected by measles was the 5 to 9 year olds followed by the 2 to 4 years age group. Additionally, among the HIV-infected children on HAART, age at initiation of HAART and duration on HAART were found

not to be significant predictors of measles. The CD4+ percentage of total lymphocytes

prior to exposure to measles was also not a significant predictor of measles illness in

HIV-infected children on HAART. Undernutrition (particularly stunting) and age were

found to be significant predictors of measles illness in the HIV-infected children on

HAART. However, the proportion of children who suffered from measles in the exposed

and unexposed groups was so low that the findings should be interpreted with caution.

Interpretation and Discussion of the Key Findings

Risk of Measles

Results of the analysis of the primary research question showed that there was no

significant difference in the risk of measles between the HIV-infected children on

HAART and HIV-uninfected children. This result is contrary to what was expected given

the potential for secondary vaccine failure among HIV-infected children previously

reported by others (i.e. Aurpibul et al., 2006; Farquhar et al., 2009; Fowlkes et al, 2011;

Polonsky et al., 2014; Rainwater-Lovett, Nkamba, Mubiana-Mbewe, Bolton-Moore, &

Moss, 2013; Tejiokem et al., 2007). However, the immunization status and particularly

the number of measles vaccine doses received were not well recorded or not known and

hence could have affected the result. Several of the children were orphans adopted by

relatives or in orphanages that did not know the immunization history of the children.

Furthermore, immunization status on this study was assessed on the routine immunization

schedule only. The medical records indicated if the children had received measles

vaccination as part of the routine immunization schedule. Hence, the number of doses

recorded for those that had received measles vaccination was one (1). There was no

record or question in the enrollment or follow up tools regarding measles vaccine doses

from mass measles vaccination campaigns, although Kampala and Wakiso had conducted

mass measles vaccination campaigns in 2003, 2006, and 2009 (Baliraine et al., 2011;

Mbabazi et al., 2009).

Among the HIV-infected children on HAART in this study, 119/223 (53%) were

reported to have been vaccinated against measles at routine immunization, with a single

dose of measles vaccine at 9 months. However, for 89/223 (40%) of the children,

immunization status was recorded as "unsure". A clinician at Nsambya HCD clarified

that since most of the children were enrolled into care when they were severely

immunosuppressed, measles vaccination was not specifically recommended for those that

had not yet received it or whose immunization status was "unsure."

Furthermore, there was no data available on the level of measles antibodies for the

study participants prior to the measles outbreak. It is possible that most of the HIV

positive children on HAART could have received measles vaccine during the mass

measles vaccination campaigns in the country in 2003, 2006 and 2009 (Baliraine et al.,

2011; Mbabazi et al., 2009). In 2003, a catch-up measles vaccination campaign was

conducted in Uganda targeting all children from 6 months to 14 years of age and attained

a coverage of 104% (Mbabazi et al., 2009).

In 2006 and 2009, follow up measles campaigns targeting children less than 5

years old were conducted, attaining coverage of more than 90% (Baliraine et al., 2011).

Given that most of the HIV-infected children on HAART in this study were older than 5

years old, it is possible that several of them could have received a dose of measles

vaccine while already on HAART, after immune reconstitution, which could have boosted their protection against measles. Melvin and Mohan (2003) and Aurpibul et al. (2010), showed that HIV-infected children on HAART, with immune reconstitution, develop protective measles antibodies when revaccinated. Many HIV-uninfected children younger than 5 years old could have also received more than one dose of measles vaccine from routine immunization and/or measles campaigns conducted in 2006 and 2009.

These immunized children were not susceptible to measles by the time of the measles outbreak, between November 2011 and June 2012. The mean routine vaccination coverage in Kampala and Wakiso was 108% and 94.8%, respectively, in the 5 years prior to the study (Ministry of Health Database (DHIS2) and the measles mass campaign coverage of Kampala and Wakiso were 72% and 81% in 2006 and 81% and 107% in 2009 respectively (Ministry of Health Database – Supplementary Immunization Activities Administrative Data). The coverage of more than 100% achieved in routine immunization in Kampala and in the mass measles vaccination campaign in Wakiso in 2009 were due to children coming from neighboring districts for immunization services in Kampala and Wakiso. This movement from other districts to Kampala and Wakiso, which are urban and peri-urban districts respectively, was mainly due to pursuit of higher quality services in these districts. The low proportion of exposed and unexposed children that suffered from measles in this study could be an indication that the number of susceptible people in the study population were lower than the numbers documented by Mupere et al (2006), Fowlkes et al (2011), and Tejiokem et al (2007). The low number of

susceptibles could be a reflection of the positive effect of the several mass measles campaigns in addition to the routine immunization (Uzicanin & Zimmerman, 2011).

With the low levels of P_0 and P_1 obtained in my and maintaining the desired odds ratio at 1.61, the minimum sample size required to have a 80% study power would be 1578 exposed and 1578 un-exposed children (a total of 3,156 children) (Epi Info 7 StatCalc). To obtain this number of children would require using several study sites within Kampala and Wakiso districts because it is not feasible to enroll them from Nsambya Hospital alone. However, Mendes and Akkartal (2008) indicated that with a difference in proportions equal to or less than 0.05, as seen on this study, increasing the sample size to 500 and above in each group would probably raise the power to only about 40%. The much lower than anticipated P_0 and P_1 pose a threat to the validity of the study results and could result in falsely not rejecting the null hypothesis. However, Cohen's h, a measure of effect size when there is a small risk difference, was computed and it revealed a small to medium effect size of 0.364, which implies that even if the difference in risk of measles among the HIV-infected and HIV-uninfected children is not statistically significant, it may be of clinical importance.

Hence in clinical monitoring and management of HIV-infected children on HAART, attention ought to be given to measles vaccination and screening to minimize the clinical risk of measles. Furthermore, the confidence interval of the risk difference was computed; zero was within the confidence interval indicating that the risk difference was not significant. Triangulation of the significance of the risk difference and the confidence interval of the risk difference indicated that the chances of falsely not

rejecting the null hypothesis were minimal. A follow up multi-site study to investigate

further the risk of measles in HIV-infected children on HAART would thus possibly

provide results with higher power, though the power may still fall short of the desired

80%.

There is need for health care workers to keep better vaccination histories of

children being seen at Nsambya Hospital in order to enable assessment of the effect of

vaccination efforts on the measles incidence. In the absence of detailed information on

vaccination status of both the exposed and unexposed children, the finding of no

difference in the risk of measles should be interpreted with caution. Furthermore, other

factors, such as malnutrition, that increase susceptibility to measles could not be adjusted

for in research question one, as the data on nutrition status was not available for HIV-

uninfected children. It is also not known whether there were HIV-infected children on

HAART who could have developed subclinical measles or measles without a skin rash as

was seen in the study by Rainwater-Lovett et al. (2013) in Zambia. However, given the

small to medium effect size, the risk difference observed may be clinically important and

thus worth further investigation, especially in sites where vaccination status is well

recorded. Page (2014), in his commentary on clinical importance of research findings

beyond statistical significance, suggested that a small to medium effect could be

clinically important especially in the clinical care of individual patients.

Age-group Most Affected by Measles among HIV-infected Children on HAART

In response to the first secondary question, the study results showed that the HIV-

infected children on HAART most affected by measles were the 5 to 9 year olds followed

by the 2 to 4 year olds. In this study, age was a significant predictor of measles among HIV-infected children on HAART, with the risk increasing with age. This result could be due to secondary vaccination failure, given that most of the children could have been reached by the different vaccination campaigns in addition to routine immunization.

Over half (14/26, or 54%) of the HIV-infected children who suffered from measles had received the measles vaccine through routine immunization, 4 (15%) had not been vaccinated, and for 8 (30%) caretakers were unsure of the vaccination status. Wirth et al. (2015) also found that in Botswana, older HIV-infected children and adolescents were more affected by measles than the younger age group, although their age groups were different from those used in this study. The authors recommend re-vaccination in HIV-infected older children and adolescents (Wirth et al., 2015).

Given the age groups most affected by measles among the HIV-infected children on HAART in Uganda, a booster dose of measles may be useful for such children before this age, after immune reconstitution, to enhance their protection against measles (Sutcliffe & Moss, 2010). Booster doses have been shown to induce a protective level of measles antibodies in children on HAART with immune reconstitution (Abzug et al., 2010; Aurpibul et al. 2010; Farquhar et al., 2009; Melvin and Mohan, 2003; Polonsky et al., 2014; Rainwater-Lovett et al., 2013; Rowson et al., 2015). Provision of a booster dose in children with immune reconstitution could be considered at 2 years of age or soon after, to ensure protection of the children from measles, given that 2 to 4 years was the second most affected age group.

Fowlkes et al. (2011) and Tejiokem et al. (2007) also showed that by 2 years of age 60 to 80% of the HIV-infected children had experienced secondary vaccination failure. Another booster dose may be given at 5 years to further protect the children from measles as they grow older. However, it may be more appropriate to include monitoring of the level of measles antibodies in the care of HIV-infected children on HAART in Uganda, as it is being done in pediatric HIV clinics in some countries (Melvin & Mohan, 2003; Rowson et al., 2015). This practice would ensure that booster doses are given to individual children with immune reconstitution when their measles antibody levels reduce to below protective level. The monitoring of antibodies could be done alongside the CD4+ and viral load monitoring and should be continuous, as some children may require even more booster doses as they grow older. Melvin and Mohan (2003) found that 30% of HIV-infected children on HAART that had developed protective measles antibodies after re-vaccination had a decline in level of antibodies by one year after re-vaccination. Aupurb et al. (2010) found that there was a median annual decay of measles antibodies of about 12% in the first 5 months post re-vaccination, which reduced subsequently to 2.7% in 3 years post vaccination.

Similar to findings by Melvin and Mohan (2003), the level of antibodies in the Aupurb et al. study (2010) still remained above protective level by 3 years, except for 2 children that lost the measles antibodies and became sero-negative. Abzug et al. (2010) showed a 14% reduction of children with protective measles antibodies post revaccination; from 89% at 8 weeks after revaccination to 75% after 4 years. Similarly, Rowson et al. (2015) showed that 81% of revaccinated HIV-infected children on HAART

in the United Kingdom developed protective measles antibodies. However, in the United

Kingdom, measles seroconversion was more likely in children with undetectable HIV

viral loads.

This finding was similar to the results obtained by Abzug et al. (2010), where low

or undetectable HIV viral load (less than 400 copies per ml) were associated with higher

level of measles antibodies post revaccination. These findings show the benefits of

revaccination in HIV-infected children on HAART who have achieved immune

reconstitution and low or undetectable viral loads. They also show that the antibodies

acquired after revaccination wane, implying that periodic monitoring of measles

antibodies should be a key component of care for children on HAART.

The results of the monitoring of measles antibody could then guide the

appropriate provision of booster doses of measles vaccine. Developing countries, like

Uganda, with high prevalence of HIV, targeting measles elimination, should consider

building the capacity for periodic measles antibody monitoring as part of pediatric HIV

care. They should also consider provision of booster doses as part of the immunization

schedule for HIV-infected children on HAART. In low resource settings, and in up-

country clinics in Uganda, where the monitoring of measles antibodies may not be

feasible in the short-term, the administration of booster doses at 2 years and 5 years of

age may be a programmatic option, provided the children have been on HAART for at

least 6 months. It has been shown that the immune system of most children on HAART is

reconstituted within 6 months of initiation of HAART (Farquhar et al., 2009; Moss,

2015). Moss (2015), in her paper to the WHO Strategic Advisory Group of Experts on

Immunization, also recommended that the committee approves measles revaccination in HIV-infected children on HAART, after immune reconstitution.

Predictors of Measles

Results from this study showed that age at initiation of HAART was not a significant predictor of measles in HIV-infected children on HAART. Although the risk of measles in children who started on HAART at age less than 9 months was about a half of those who started on HAART at 9 months and above, the result was not statistically significant. This finding is consistent with the one by Aurpibul et al. (2006), which showed that age at initiation of HAART was not a significant predictor of level of measles antibodies. The finding in this study was not consistent with the findings by Pensieroso et al. (2009), which showed that initiation of HAART early in life, before measles vaccination, could prevent secondary vaccination failure and thus lower the risk of measles significantly in HIV-infected children. However, the children started on HAART prior to 9 months in this study already had deteriorated immune system, with CD4+ percentage less than 15%, different from the study by Pensieroso et al. (2009) where the immune system of the children had not yet deteriorated.

Most of the children in the study that were enrolled into care before 9 months, had not reached the threshold for initiation of HAART at Nsambya HCD, of CD4+ percentage of 15% or less, by 9 months and were thus started on HAART after 9 months. Furthermore, the majority of children were enrolled in care late, after their first year (mean age at initiation of HAART was 5.67). As there were very few children (10) started on HAART prior to 9 months, the finding from this study showing that age at

initiation of HAART was not a predictor of measles in HIV-infected children on HAART

may not be conclusive. The recent revision of anti-retroviral therapy guidelines

recommends initiation of HAART as soon as any child is confirmed as HIV-infected,

regardless of the CD4+ percentage (Uganda Ministry of Health, 2013). As infant

diagnosis of HIV as early as 6 weeks of age, and HAART have become more accessible

in Uganda, many children are likely to be initiated on HAART before 9 months. This

would enable better assessment of the effect of age at initiation of HAART on measles

antibodies and measles susceptibility. Similarly, the duration on HAART was not a

predictor of the risk of measles in this study. This finding was consistent with findings by

Aurpibul et al. (2006) and Tejiokem et al. (2007), which also showed that duration of

HAART was not a predictor of measles antibody level, and thus not a predictor of risk of

measles.

To investigate whether low nutrition status was a significant predictor of measles

in HIV-infected children on HAART, in this study I used three measures of nutrition

status including height for age, weight for age, and weight for height. Undernutrition on

the height for age parameter in a child (stunting) is an indication of chronic malnutrition,

which can result in primary or secondary measles vaccine failure, and could thus increase

the risk of measles. Undernutrition on the weight for height or body mass index for age

parameter (wasting) in a child is an indication of acute malnutrition. Wasting is

associated with high mortality among children with measles, especially in the presence of

Vitamin A deficiency (Franca et al., 2009; Katona & Katona-Apte, 2008). Undernutrition

on the weight for age parameter (underweight) shows disharmony in both linear growth

and body proportion and may increase risk of measles illness and mortality. The results of this study showed that undernutrition on body mass index for age (wasting) and undernutrition based on weight for age measurements (underweight) were not significant predictors of measles in the exposed children. However, undernutrition for height for age parameter (stunting) was a significant predictor of measles in HIV-infected children on HAART. This result is consistent with the finding by Waibale et al. (1999) who showed that it was malnutrition (stunting) and not HIV per se that was associated with loss of measles anti-bodies in children in Uganda.

Regarding wasting, the finding from this study is not consistent with the result by Kizito et al. (2013), which showed that infant wasting was one of the significant factors that were contributing to measles vaccine failure in HIV-infected children in Uganda. Kizito et al. (2013) analyzed the level of measles antibodies among children one year old, only 3 months after routine immunization. It is thus likely that infant wasting contributed to primary vaccine failure and not secondary vaccine failure in these children, rendering them susceptible to measles. Furthermore, the finding was contrary to the one by Korutaro et al. (2013), which showed that wasting was a significant factor associated with measles illness and death in HIV-infected children in Uganda. Pertaining to underweight, the result of this study is not consistent with the one by Korutalo et al. (2013) that showed that underweight was also a significant factor associated with measles illness and death. Whereas findings from this study as well as those by Kizito et al. (2013), Waibale et al. (1999), and Korutaro et al. (2013) all show malnutrition as a significant predictor of measles in HIV-infected children in Uganda, different parameters

of malnutrition are significant in the different studies. However, these findings indicate that low nutrition status is generally a predictor of measles in HIV-infected children in Uganda. Stunting showed a much higher odds ratio in this study than any other forms of malnutrition, a possible indication that stunting could be posing the highest risk of measles in the HIV-infected children. In studies conducted by Tejiokem et al. (2007) in Cameroon and Central African Republic and Aurpibul et al. (2006) in Thailand, using different methods from my study, the findings showed that malnutrition was not a significant predictor of measles antibodies. Thus, malnutrition may be a specifically unique and more prevalent problem in Uganda than in other countries, affecting vaccine effectiveness in Ugandan children.

In my study, stunting was present in over 40% of children on HAART. Similarly, stunting is a national problem in Uganda with about 38% of children less than 5 years stunted, regardless of HIV status (Uganda Bureau of Statistics (UBOS) & Macro International Inc., 2007). In Kampala region, where the Kampala and Wakiso districts are situated, 22% of children are stunted, regardless of HIV status (Uganda Bureau of Statistics (UBOS) & Macro International Inc., 2007), a proportion lower than what I found among HIV-infected children on HAART in this study. Height for age (stunting) could not be assessed in the unexposed children as the variables were not recorded in the medical records in Nsambya OPD. However, it is possible that stunting was also a significant predictor of measles in this group and a potential confounder of the measles risk difference between HIV-infected and HIV-uninfected children.

All forms of malnutrition prior to measles vaccination can result in primary vaccine failure while malnutrition after measles vaccination can result in suppression of body immunity and secondary vaccine failure (Franca et al., 2009; Kizito et al., 2013; Waibale et al., 1999). The finding in this study that a form of malnutrition (stunting) was a significant predictor of measles in HIV-infected children on HAART is also in accordance to the proximate determinant framework adapted for this study. In the proximate determinants framework, malnutrition is a proximate determinant of measles and secondary vaccine failure contributed to by malnutrition is an immediate determinant of measles. This underscores the need for stronger nutrition program in Uganda targeting reduction of stunting among all children. This will not only benefit HIV-infected children but all children, and may contribute to the attainment of measles elimination. Since malnutrition is an underlying cause in about 50% of children deaths (Rice, Sacco, Hyder, & Black, 2000), a strong nutritional program could produce a reduction in mortality among children under 5 years of age. For HIV-infected children on HAART, it is even more important to integrate a strong nutrition education, monitoring, and supplementation component in their specialized HAART clinics.

In this study, I also investigated whether low CD4+ prior to exposure to measles was a significant predictor of measles. Given that CD4+ count/percentage is a proxy of the level of immunity, it would be expected that a low CD4+ count/percentage would be associated with high risk of measles and high CD4+ count/percentage would be protective against measles. However, consistent with the findings by Aurpibul et al. (2006) and Abzug et al. (2010), the results in my study show that CD4+ count/percentage

prior to measles or measles outbreak, a measure of the level of immunity of the child prior to exposure to measles virus, was not a significant predictor of measles in HIV-infected children on HAART. Abzug et al. (2010), showed that it was the HIV viral load and not the CD4+ count/percentage that was a predictor of measles antibodies. However, in this study, I could not investigate HIV viral load as a predictor of measles in exposed children as viral load was not routinely assessed by the HCD for all HIV+ children during the period covered by the study. Due to limited resources, HIV viral load was only being assessed for children who were not responding well to HAART or where emerging resistance to HAART regimen was suspected.

My study finding is not consistent with that of Tejiokem et al. (2007) where low CD4+ percentage (less than 25%) was associated with low and unprotective measles antibodies, hence higher risk of measles. Sutcliffe and Moss (2010) suggest that treatment with HAART results in immune system recovery that involves production of naïve T cells and probably not an increase in memory cells. It is uncertain whether the remaining B memory cells maintain or regain their functionality in people on HAART (Pensieroso et al., 2009). This may be the reason the increase in CD4+ is not a predictor of high and protective measles antibodies and low measles risk. Further research is needed to investigate the relationship between HIV viral load and risk of measles in Uganda given that viral load has been incorporated in routine monitoring of HAART in all HIV-infected people in accordance to the guidelines from WHO (WHO, 2014b).

My findings are in harmony with the proximate determinants framework adapted for this study. A weak nutrition education component of the HIV care programme is an

underlying determinant that results in chronic malnutrition of HIV-infected children. In this study, chronic malnutrition (stunting) was found to be a significant predictor of measles in HIV-infected children on HAART. Thus chronic malnutrition (stunting) is a proximate determinant of measles in HIV-infected children on HAART; it results in secondary measles vaccine failure (immediate determinant) and thus makes the children susceptible to measles. Similarly, treatment with HAART is also a proximate determinant as it contributes to longer survival of the children. Results of this study showed that older HIV-infected children on HAART were at higher risk of measles, necessitating booster doses of measles vaccine at 2 years and 5 years. HIV infection is another proximate determinant; though not statistically significant, HIV-infected children on HAART had a 41% higher risk of measles than HIV-uninfected children. The finding that CD4+ count prior to exposure to measles was not a significant predictor of measles was however contrary to the adapted proximate determinant framework where low CD4+ is an immediate determinant of measles in HIV-infected children on HAART. Instead, HIV viral load may be more of an immediate determinant than CD4+ count prior to measles exposure (Abzug et al., 2010). However, HIV viral load was not examined in this study.

Limitations of the Study Findings

This retrospective cohort study used past medical records that lacked data on some variables. Thus, the analysis in some questions had to use fewer numbers of children than the total sample. For one secondary question, the number of children included in the analysis was less than the minimum sample size estimated a priori for that group. This limitation contributed to the results not being conclusive. Some important

variables like immunization status were not well recorded and thus could not be included in the analysis. In the absence of data on immunization status and number of measles doses received, these variables were excluded from the multivariate analysis. Without data on measles vaccination, number of measles vaccine doses received, and level of measles antibodies of exposed and unexposed children, the results of the study, especially for the primary research question, may not be conclusive. However, efforts were made to manually compute missing variables where possible e.g. the CD4+ percentage, and make use of the best available data to answer the study questions. Where necessary the data analysis plan was adjusted due to limitations in data available. For example, immunization status was excluded in the binomial logistic regression in the primary research question. Furthermore, instead of forward stepwise logistic regression simultaneous logistic regression was undertaken in the analysis for underweight as a possible predictor of measles in HIV-infected children on HAART. This was due to the total number of children with this variable being less than the recommended ratio of 20 cases to one variable for stepwise logistic regression.

The study did not include viral load as a variable due to the paucity of data on this variable during the study period. Capacity to conduct routine viral load monitoring was not available at HCD. Furthermore, the data on level of measles antibody by the time of exposure to measles virus was not available for this study as it is not routinely monitored. Yet such data would have given more information on susceptibility of the children to measles by the time of exposure to measles virus in the measles outbreak of November 2011 to June 2012.

The proportion of exposed and unexposed children that suffered from measles was much lower than anticipated in the study proposal. Whereas this could be an indication of increased population immunity, it had a negative effect on the power of the study. A much larger sample would be required to increase the power of the study, however this was not logistically possible. Nevertheless, several analytic procedures including Cohen's *h*, a measure of effect size when there is a risk difference of 0.05 or less, and confidence intervals of the risk difference were used to understand further the risk of measles and to ascertain further whether the null hypothesis should not be rejected.

In this study, although exposed and unexposed were from the same age group (2 to 15 years), there was no matching by actual age at time of measles. The exposed and unexposed groups had different age structure, with unexposed children being significantly younger than the exposed children were. However, adjustment for age was done in multivariate analysis to enhance comparability between the two groups. In spite of the study sample being representative of the population from which it was drawn, due to the small numbers of the children who suffered from measles among exposed and unexposed children, and the resultant low power limitations, the results of this study are not generalizable beyond the study site.

Recommendations

Monitoring of level of measles antibodies should be incorporated into the routine care of HIV-infected children regardless of HAART status. This monitoring will enable timely detection of children whose level of measles antibodies reduce below protective

level. These children would then be considered for revaccination if their immune system has been reconstituted. The immunization policy for the HIV-infected children on HAART should be reviewed to include a booster dose of measles vaccine when the measles antibodies are just below protective level, where routine monitoring of measles antibodies is feasible. In low resource settings, where routine monitoring of measles antibodies is not possible, consideration should be given to administration of booster doses of measles vaccine programmatically at 2 and 5 years of age. This will reduce susceptibility and risk of measles in HIV-infected children as they grow older and could enhance attainment of measles elimination targets by decreasing the pools of measles susceptible children created by secondary vaccine failure.

A strong nutrition and supplementation program to prevent chronic malnutrition should be integrated in pediatric HIV care clinics in Uganda including Nsambya HCD. Nutrition monitoring and education should indeed be incorporated in all pediatric clinics and outreaches to reduce on the magnitude of chronic malnutrition among Ugandan children. These strategies will contribute greatly to the ongoing national efforts to reduce under-five morbidity and mortality.

In order to facilitate high quality care of patients, there is need to improve completeness of medical information in the records. The standards of recording of key information including immunization status in medical records should be emphasized in all health facilities in accordance to the health management and information guidelines in Uganda (Ministry of Health Resource Center, 2010). Appropriate recording of key information about patients will enhance quality of care and follow up of the patients. For

example, children recorded as not immunized would be followed up and provided with the appropriate vaccination in the OPD or while on the ward. With a level of incompleteness of medical records as found in this study, conducted in one of the best hospitals in Kampala city, quality of care may be compromised. The Ministry of Health should inform and supervise hospitals and clinicians to improve on completion of medical records.

A multi-center study with much bigger sample size is recommended to further understand the risk difference between HIV-infected children on HAART and HIV-uninfected children, and whether age at initiation of HAART, low CD4+ prior to measles exposure, and low nutrition status are predictors of measles. Furthermore, with the new guidelines on HAART initiation in children immediately after they are diagnosed as HIV-infected, it would be good to monitor measles antibody levels of the children who are initiated on HAART early in life. This would generate information on whether secondary vaccine failure occurs in the HIV-infected children on HAART at rates different from HIV-uninfected children.

Implications for Positive Social Change

The results of my study contribute to the knowledge base about the risk factors for measles in HIV-infected children on HAART. The finding that chronic malnutrition (stunting) is a significant predictor of increased risk of measles among HIV-infected children on HAART, could be used to advocate for a stronger nutrition program for these children. A stronger nutrition program, initiated right from birth, will reduce the prevalence of chronic malnutrition and secondary vaccine failure among the HIV-

infected children on HAART. The reduction in malnutrition and secondary vaccine failure would hence contribute to a reduction of pools of measles susceptible children that could maintain transmission of measles within communities and hamper measles elimination efforts.

Furthermore, the finding from my study of the age group of HIV-infected children on HAART most affected by measles provides new information on the appropriate ages of revaccination of these children in Uganda. This information could be used to advocate for and inform a change in vaccination policy and practice for HIV-infected children on HAART in Uganda. The finding about the age groups most affected by measles among the HIV-infected children and hence the appropriate age for revaccination could provide more evidence for the advocacy with SAGE by Moss (2015) for the need for booster doses of measles vaccine for HIV-infected children on HAART. The change in vaccination policy would ensure that the HIV-infected children on HAART are protected from measles and the increased mortality associated with the disease among these children.

The prevention of measles mortality in these children would contribute to reduction in childhood mortality rate in Uganda. The reduction in susceptibility to measles illness and mortality among HIV-infected children on HAART accruing from adoption of the above programmatic, policy and practice changes would contribute significantly to positive social change among the HIV-infected children and their families; it would ensure that such children, whose survival and quality of life are improved by HAART are protected from and not lost to measles at an older age. When

the pool of HIV-infected children on HAART that are susceptible to measles is reduced, this could enhance feasibility of attainment of measles elimination in the districts of Wakiso and Kampala and in Uganda in general.

Conclusions

From results of this study, there may be no significant measles risk difference between HIV-infected children on HAART and HIV-uninfected children in the same age group. However, the small to medium effect size indicates that the difference, nonsignificant as it may be, may be of clinical importance. Thus, clinicians treating HIV-infected children on HAART need to take special interest in protecting the children from measles by ensuring that their measles antibodies are kept above protective level through an appropriate vaccination strategy. Results from this study are not conclusive and are interpreted with caution due to the low numbers of children that suffered from measles in the study period, the lack of adequate information on immunization status, and the resultant low post-hoc power of the study. A much bigger sample size would be needed to achieve a higher study power. However, given the very low difference between P_0 and P_1, even increasing the sample size to 500 and above on each group would probably raise the power of the study to not more than 40% (Mendes & Akkartal, 2008). Analytical measures including using Cohen's h, a measure of effect size when the difference in proportions is less than 0.05, and the confidence interval of the risk difference were used to make inference on the risk of measles among HIV-infected children on HAART compared to uninfected children in the same age group. Among HIV-infected children on HAART, those 5 to 9 years old were most affected by measles followed by children 2 to

4 years old indicating the need for booster doses at 2 years and at 5 years to reduce the risk of measles in children on HAART with immune reconstitution.

Pertaining to the factors that were predictors of measles in HIV-infected children on HAART, stunting emerged as a significant predictor of measles in these children. Age was another significant predictor of measles in the HIV-infected children on HAART in this study, with the measles risk increasing with age. This finding is consistent with findings in several studies that showed waning measles antibodies as HIV-infected children grew older. CD4+ count prior to exposure to measles, and age at initiation of HAART were found not significant predictors of measles in this study. However, all these findings were also not conclusive due to the low numbers of children who developed measles among the study participants. A bigger multi-site study, involving interviewing of caretakers and children on details of vaccination status, including other variables like viral load and level of measles antibodies prior to exposure to measles would provide more information to complement the findings of this study.

Given the significance of chronic malnutrition (stunting) as a predictor of measles among HIV-infected children on HAART, a stronger nutrition education and care component, integrated into the HIV care programme, is recommended. Furthermore, incorporating monitoring of measles antibodies with the HIV care programme is recommended to ensure that the trend of children's measles antibodies is followed and a booster dose of vaccine provided when the antibody level reduces to just below the protective level. This will reduce susceptibility of HIV-infected children on HAART to measles. Additionally, given the age group most affected by measles among the HIV-

infected children on HAART, booster doses of measles vaccine are also recommended at 2 years and 5 years in low resource settings where routine monitoring of measles antibodies may not be feasible, if the child has been on HAART for at least 6 months.

The findings of this study contribute to better understanding of the risk factors for measles in HIV-infected children on HAART. The results also provide evidence for advocacy for a stronger nutrition education and care programme for HIV-infected children in order to prevent malnutrition and the related vaccine failure and high risk of measles among these children. Furthermore, the study findings provide new information on the possible age for revaccination of HIV-infected children on HAART that contributes evidence to the advocacy on the need for and scheduling of revaccination of HIV-infected children on HAART. The results of my study thus provide evidence to inform vaccination practice and care for HIV-infected children on HAART that could cause positive social change for these children, their families, and Uganda in general. Protecting the HIV-infected children on HAART against measles enables them to survive longer and prevents unnecessary deaths due to measles among these children. This would alleviate the social and economic impact of premature deaths on many families that have HIV-infected children on HAART. A vaccination policy that ensures that these children are not susceptible to measles will enhance attainment of measles elimination by the country as it avoids accumulation of pools of susceptible HIV-infected children that would otherwise maintain measles transmission within communities and hinder attainment of measles elimination.

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Appendix A: Data Collection Tool

Data Collection Tool

Study Identification Number _____Clinic Number _____   Exposed ___

Unexposed___

Demographic and Contact Details

Age: ____years ___months   Sex: ___Female

    ___Male

Residential Address:

District: _____   Sub-county: _____

    Village:_____

Vaccination History

Has child ever been vaccinated against measles? ____Yes ____ No ____ Not Sure

(If No or Not Sure, please move to C below)

If yes, how many doses of measles vaccine has the child ever been given? __1 __2 __3

and above __ Unknown

Measles vaccination history

Measles vaccine dose	Age at vaccination (Years and months)	Mode of delivery (Routine immunization or vaccination Campaign)	Validated on Immunization card (Yes or No)
1			
2			
3 and above			
Unknown			

History of Measles

Did the child suffer from measles illness during or after the measles outbreak of

November 2011 to June 2012? ___ Yes   ___ No ___ Not sure *(If No or Not Sure,*

proceed to D)

If yes:

Age at measles illness: ____years ____months

How was measles confirmed? ____Clinically   ____ By Laboratory

What signs and symptoms did the child have?

Fever   ____Yes ___No   Cough ____Yes ____No

Conjunctivitis (Red eyes)___Yes ___No

Skin Rash ___Yes ___No   Coryza (running nose) ___Yes ___No

Oral sores ___Yes ___No   Others (specify)_____

Did child experience any of the following complications?

Pneumonia ___Yes ___No   LTB (Croup) ___Yes ___No Candidiasis (oral thrush)

__Yes __No

Malnutrition ___Yes ___No Others (specify)

_____

For how long was the child ill with measles? _____days

For Exposed Children

What was the child's CD4+ count/percentage before he/she developed measles _____

What was the child's weight and height before he/she developed measles? Weight _____

Height _____

HAART Initiation

Is child on HAART? _____Yes ___No

At what age was the child started on HAART? _____Years ___Months

Has child had any interruptions in HAART since initiation? ___Yes ___No

If yes, for how long? ____Years ____Months

What was the child's last CD4+ count/percentage before initiating HAART? _____

Date of last CD4+ results before HAART __/__/____

Nutrition Status

For Exposed Children with history of measles

What was the weight of the child just before measles? _____Kg

What was the height of the child just before measles? _____Cm

Weight for height Z-Score _____   Nutrition status

Category_____

Height for age Z-Score _____   Nutrition status

Category_____

Weight for age Z-Score _____

Nutrition status Category_____

Appendix B: Description of Variables in the Study

Variable	Description
Age	Number of years and months by time of exposure
Sex	Whether child is a male of a female
History of measles vaccination	Whether the child got measles vaccination prior to measles outbreak
Number of measles vaccine doses	Numbers of measles vaccine doses received by the child prior to the measles outbreak
Had measles illness	Whether the child got measles or not during the measles outbreak
Age at measles illness	How old (years and months) the child was when developed measles; only applicable for children who developed measles
CD4+ count before measles illness or outbreak	The number of CD4+ cells/ml in HIV-infected children. For the exposed children (suffered from measles), this was the last CD4+ count (within 3 to 6 months) prior to measles illness. For those without measles, this was the last CD4+ count (within 3 to 6 months) prior to November 1 2011 (estimated date of the measles outbreak start). Applicable to exposed children only.
CD4+ percentage before measles	The absolute CD4+ number was transformed into percentage by dividing CD4+ number by total lymphocytes and multiplying by 100. The CD4+ percentage is a measure of the child's immune status prior to exposure to measles and was applicable only to HIV-infected children on HAART.

illness or
outbreak

Variable	Description
Immune status before measles illness or outbreak	Based on the CD4+ percentage, the child's immune status was categorized as either immune suppressed (CD4+ percentage less than 25%) or normal immunity (CD4+ percentage of 25% and above). This variable was used in the analysis of whether low CD4+ percentage was a predictor of measles in HIV-infected children on HAART.
HAART Treatment	Indicated whether the child was on HIV treatment or not prior to measles outbreak; this variable was applicable to HIV-infected children only.
Age at HAART Initiation (Continuous variable)	How old (number of years and months) the child was when the child was started on HIV treatment. This variable was used as a covariate in multivariate analysis for the secondary questions investigating whether nutrition status or low CD4+ percentage were predictors of measles in the exposed children..
Age at initiation of HAART (Categorical variable)	The age at HAART initiation was transformed into a categorical variable creating 2 age groups: less than 9 months and 9 months and above. This categorical variable was used as an independent risk factor for measles in exposed children in question 3.
Duration on HAART (Continuous variable)	The length of the period the child had been on HIV treatment. The duration on HIV treatment was computed by subtracting the age at initiation of HAART from the age at time of exposure to measles. This variable was used as a covariate in the analysis of age at HAART initiation as a risk factor for measles in exposed children.

Variable	Description
Duration on HAART (Categorical variable)	This variable was categorized into duration on HAART of less than 2 years, and 2 years and above and used as an independent variable in the analysis in question 3.
CD4+ count at HAART initiation	The absolute number of CD4+ cells/ml. This is a measure of the child's immune status prior to initiation on to HAART and was applicable only to HIV-infected children.
CD4+ percentage prior to HAART initiation	The absolute CD4+ number was transformed into percentage by dividing CD4+ by total lymphocytes and multiplying by 100. It was used as a covariate in the analysis in question 3.
Immune status prior to HAART initiation	Based on the CD4+ percentage prior to HAART initiation, the immune status of the exposed children was categorized into severely immune suppressed (less than 15%) and not yet severely immune suppressed (15% and above) during analysis. This was applicable only to HIV-infected children on HAART and was used as an independent variable in the analysis on whether low CD4+ percentage was a predictor of measles in exposed children .
Weight	Weight of the child before measles illness or measles outbreak in kilograms
Height	Height of the child before measles illness or measles outbreak in centimeters

Variable	Description
Weight for age	Measures whether the child has normal weight for age (normal nutrition) or is under-weight for age (undernutrition). The weight for age Z-score was used to categorize nutrition status as either normal status (Z-score less than 2 and more than -2) or undernutrition (Z score less than -2) in the analysis.
Underweight	Refers to undernutrition on the weight for age parameter. Underweight shows disharmony in both linear growth and body proportion. This variable was used as an independent variable in the analysis on whether nutrition status was a predictor of measles in exposed children.
Weight for height	Measures whether the child has the expected weight for his/her age (normal nutrition) or weighs less than expected for his/her height, that is, child is wasted (undernutrition). The Z-score for weight for height was applicable for only children less than five years and was used to categorize nutrition status as either normal nutrition status (Z-score less than 2 and more than -2) or undernutrition (Z score less than -2).
Body Mass Index (BMI) for age	BMI for age is a measure of whether a child older than 5 years has the expected weight for his/her age (normal nutrition) or weighs less than expected for his/her height (undernutrition). The Z-score for BMI for age was applicable for only children older than five years and was used to categorize nutrition status as either normal nutrition status (Z-score less than 2 and more than -2) or undernutrition (Z score less than -2)
Wasting	Refers to Under nutrition on the weight for height and BMI for age parameters. Wasting is an indication low weight in relation to a child's height and is a result of recent or acute malnutrition (Shetty, n.d). This variable was used as an independent variable in the analysis on whether nutrition status was a predictor of measles in exposed children.

Variable	Description
Height for age	Height for age is a measure of whether the child has appropriate height for his/her age (normal nutrition) or height is less than expected at his/her age (Under Nutrition). The height for age Z-score was used to categorize nutrition status as either normal nutrition status (Z-score less than 2 and more than -2) or undernutrition (Z score less than -2).
Stunting	Refers to under nutrition on the height for age parameter and is an indication of chronic malnutrition (Shetty, n.d). This variable was used as an independent variable in the analysis on whether nutrition status was a predictor of measles in exposed children.

Appendix C: Codes and Level of Measurement for Variables in this Study

Variable	Codes	Level of measurement
Age		Continuous
Sex	1 = Male	Nominal
	2 = Female	
History of measles vaccination	1 = Yes	Nominal
	2 = No	
Number of measles vaccine doses	1 = 1 dose	Ratio
	2 = 2 doses	
	3 = 3 doses and above	
	9 = Unknown	
Had measles illness	1 = Yes	Nominal
	2 = No	
Age at measles illness		Continuous
CD4+ count before measles illness or outbreak		Continuous
CD4+ percentage before measles illness or outbreak		Continuous
Immune status before measles illness or outbreak	1 = Immune suppressed (CD4+ percentage less than 25%)	Nominal
	2 = Normal immunity (CD4+ percentage of 25% and above).	
HAART Treatment	1 = Yes	Nominal
	2 = No	

Variable	Codes	Level of measurement
Age at HAART Initiation		Continuous
Age at initiation of HAART (Categorical variable)	1 = Less than 9 months 2 = 9 months and above	Nominal
Duration on HAART		Continuous
Duration on HAART (Categorical variable)	1 = Less than 2 years 2 = 2 years and above	Nominal
CD4+ count at HAART initiation		Continuous
CD4+ percentage prior to HAART initiation		Continuous
Immune status prior to HAART initiation	1 = Severely immune suppressed (CD4+ percentage prior to HAART initiation less than 15%) 2 - Not yet severely immune suppressed (CD4+ percentage prior to HAART initiation of 15% and above)	Nominal
Weight		Ratio
Height		Ratio
Nutrition Status (Weight for age)	1 = Under Nutrition (Z-Score less than -2) 2 = Normal Nutrition (Z-Score more than -2)	Nominal
Underweight	1 = Yes (Under Nutrition on Weight for Age) 0 = No (Normal Nutrition on Weight for Age)	Nominal
Nutrition Status (Weight for height)	1 = Under Nutrition (Z-Score less than -2) 2 = Normal Nutrition (Z-Score more than -2)	Nominal
Nutrition Status (BMI for age)	1 = Under Nutrition (Z-Score less than -2) 2 = Normal Nutrition (Z-Score more than -2)	Nominal

Variable	Codes	Level of measurement
Wasting	1 = Yes (Under Nutrition on Weight for Height or BMI for Age)	Nominal
	0 = No (Normal Nutrition on Weight for Height or BMI for Age)	
Nutrition status (Height for age)	1 = Under Nutrition (Z-Score less than -2) 2 = Normal Nutrition (Z-Score more than -2)	Nominal
Stunting	1 = Yes (Under Nutrition on Height for Age) 0 = No (Normal Nutrition on Height for Age)	Nominal