Sustained responses to measles revaccination among Kenyan HIV-infected children on antiretroviral therapy

Laura Newman

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2014

Reading Committee:
Carey Farquhar, Chair
Anna Wald
Jonathan Gorstein

Program Authorized to Offer Degree:
Epidemiology

University of Washington

Abstract

Sustained responses to measles revaccination among
Kenyan HIV-infected children on antiretroviral therapy

Laura Newman

Chair of the Supervisory Committee: Carey Farquhar
Professor
Epidemiology, Global Health, and Allergy and Infectious Disease

HIV-infected children have reduced rates of measles antibody production and immunologic memory after measles vaccination in the absence of antiretroviral therapy (ART). In this study, Kenyan HIV-infected children 15 months to 12 years of age on ART received an additional measles vaccine. Measles antibody concentrations were determined by enzyme-linked immunosorbent assay (ELISA) at enrollment, one month, and 12 months post revaccination. At enrollment, 125 (54%) of 232 study participants had protective levels of measles antibody. Seropositivity increased to 98% at one month post revaccination and 70% at 12 months post revaccination. Seroconversion and sustained seropositivity among those who were seronegative at enrollment was 37% at 12 months post revaccination. Low HIV viral load and increased height-for-age z-score were associated with sustained measles vaccination responses. Measles revaccination conferred a sustained antibody response in HIV-infected children receiving ART, especially those who had suppressed levels of HIV virus.

Table of Contents

Introduction

Despite recent advances in reducing overall measles incidence in Africa [1], a number of large and deadly measles outbreaks have occurred in countries with high adult and child HIV prevalence [2]. One proposed explanation for this is that, for HIV-infected infants, a single measles vaccine at 9 months of age has a response rate between 25% and 88% [3-5]. Further, even among those HIV infected infants with an adequate initial response, fewer than 50% maintain protective antibody levels after one year [3, 4]. Vaccination after initiation of antiretroviral therapy (ART) may yield satisfactory response, however ART is not always initiated before age one and thus HIV-infected children may not receive measles vaccination when it may be more effective.

Models predict an increase in prevalence of measles among HIV-infected and uninfected children as ART becomes more widely available, thus extending the life expectancy of HIV-infected children. This would be a significant reversal of recent gains in reducing childhood morbidity and mortality due to measles [6]. In areas where resources are not limited, an approach has been to provide ART and then revaccinate HIV-infected children once their immune systems have reconstituted [7, 8]. However, in a recent position paper on measles vaccination among HIV-infected children in resource-limited settings, the World Health Organization (WHO) stated that there was insufficient data about the effect of ART on measles antibody response due to a lack of studies on the subject, and concluded that there was not enough information to create a policy for revaccination against measles [9].

A related concern that could reduce measles immunization response rates in HIV-infected children is malnutrition[10]. Undernutrition during childhood growth and development can impair development of the immune system[11]. Chronic malnourishment, best measured by height-for-age z-score stunting, affects both acquired immunity and innate host defense mechanisms by impairing thymic development, T cell differentiation, T cell expansion, T cell

memory, and macrophage activation[12]. Therefore, initial and sustained measles antibody response may be reduced in malnourished children. It is well established that vitamin A plays an influential role in the regulation of immune responses [13-17]. Vitamin A supplementation, an inexpensive and easily implemented intervention, may improve measles antibody response [18, 19], though there has been conflicting evidence about the degree of the benefit [20, 21]. These previous studies were in HIV-uninfected child cohorts, where measles seroconversion rates were high, with small differences in seroconversion between supplemented and unsupplemented groups. In HIV-infected children, where seroconversion rate and sustained antibody response are much lower, nutrition deficiencies may play a greater role.

This is the largest study to evaluate the effectiveness of measles revaccination and the impact of nutritional status among HIV-infected children on ART. Previous studies on measles antibody response in HIV-infected children were conducted in sub-Saharan Africa before ART was widely available to children, or done in smaller cohorts. This study directly addresses the need for a large, long-term follow up study of measles revaccination in the vulnerable population of treated HIV-infected children. Results of the study will contribute to the development of WHO guidelines and have the potential to significantly reduce the burden of measles in low resource settings.

The Aims of this dissertation were:

1. To determine the prevalence of measles seropositivity in treated, HIV-infected children, and test whether measles revaccination results in a sustained protective measles antibody response at 12 months after revaccination. *A sustained protective measles antibody response was defined as a measles IgG antibody titer of ≥200 mIU/ml at 12 months after revaccination.*

2. To define baseline demographic and immunologic correlates associated with a sustained protective measles antibody response at 12 months after revaccination. *Potential demographic correlates included age and gender. Potential immunologic correlates included CD4%, and time on ART.*

3. To determine whether nutritional status at time of revaccination is associated with sustained protective measles antibody response at 12 months after revaccination. *Poor nutritional status is defined as a) retinol binding protein (RBP) <0.70 µmol/L and b) chronic malnutrition as measured by height-for-age z-score.*

Study Population

This study enrolled 232 children aged >15 months to 12 years who were infected with HIV and on ART. Children were recruited from the Comprehensive HIV Care Centre at Kenyatta National Hospital in Nairobi, Kenya, during routine HIV care visits. Eligibility criteria for children included HIV-1 infection confirmed by HIV-1/2 ELISA and/or PCR, on ART, aged ≥15 months and ≤12 years, CD4% ≥15%, having a caregiver who was able and willing to provide informed consent and adequate locator information, and the intention for caregiver and child to remain in Nairobi for the next two years. Exclusion criteria included a history of

adverse reaction to measles vaccination, an allergy to gelatin or neomycin, hospitalization within last two weeks, a blood or plasma transfusion within last six months, or receipt of immune globulin within the last six months.

Outline of Chapters

The two chapters that constitute this dissertation describe the three aims of this study. The first chapter describes results from the Aim 1: demographic and measles antibody titer data collected at the enrollment visit, before measles revaccination took place. This first chapter provides an understanding of measles antibody levels in the population of HIV-infected children on ART in Nairobi, and identifies those at greatest risk of initial measles vaccine failure.

The second chapter describes results from Aims 2 and 3. It provides a description of the change in measles antibody titers at one month and 12 months after measles revaccination. This chapter also examines demographic, immunological, and nutritional correlates of association for the response to revaccination by 12 months after measles revaccination.

Chapter 1: Measles Seropositivity in HIV-infected Kenyan Children on Antiretroviral Therapy

INTRODUCTION

Despite recent advances in reducing overall measles incidence in Africa, a number of measles outbreaks have occurred in countries with high HIV prevalence [2]. Poor responses to measles vaccination in HIV-infected individuals not on treatment may pose a threat to herd immunity in countries with a large HIV burden. There is evidence that antiretroviral therapy (ART) initiation alone is not sufficient for restoring measles antibody titers. A study in ART-naïve Kenyan children found 33% had positive measles IgG antibody at baseline; after six months of ART only 42% had positive measles IgG antibody in the absence of measles revaccination [3]. These data in ART-naïve children may not be generalizable to children on ART for longer durations or to those initiating ART prior to receiving measles vaccine. Data are lacking for these two populations and predictors are unknown for measles IgG seropositivity in immune reconstituted HIV-infected children.

In this report we describe the prevalence and correlates of measles IgG seropositivity in treated, immune reconstituted HIV-infected children as part of a study assessing the rationale and benefits of revaccinating children in HIV treatment programs.

METHODS

Study participants and procedures

HIV-infected children aged ≥15 months to 12 years and their caretakers were recruited from the Comprehensive Care Centre at Kenyatta National Hospital (KNH) in Nairobi, Kenya from May 2011 until January 2013. Eligibility criteria included current treatment with ART and CD4% ≥15. Written informed consent was obtained from study participants and/or their caretakers and the study was approved by the KNH/University of Nairobi (UON) Ethics Review Committee and the University of Washington Human Subjects Division.

At enrollment, a questionnaire was administered to caregivers to obtain social and demographic data. Each child was examined, and HIV diagnosis, treatment, and past medical history were obtained from medical charts. Caregivers were asked to present the child's immunization card documenting all prior vaccinations, or report immunization history verbally if the card was unavailable. Blood was obtained to determine measles IgG antibody titers, CD4%, and complete blood count. Measles vaccine, provided by the Kenya Ministry of Health, was administered to each child at the enrollment visit.

Laboratory Assays

Laboratory assays were performed in the UON Department of Paediatrics Laboratory. Qualitative detection and quantitative determination of measles IgG antibodies were performed using an enzyme-linked immunosorbent assay (ELISA) (Enzygnost, Germany), with internal quality controls performed for each run. Optical density (OD) values were interpreted as negative if <0.1, equivocal if 0.1 – 0.2, and positive if >0.2. Equivocal samples were re-run in duplicate to confirm the result. OD results >0.1 were converted into measles-specific IgG mIU/mL using manufacturer's calculations based on the First International World Health Organization (WHO) Reference Preparation Standards. Measles seropositivity was defined as an IgG titer ≥ 200 mIU/mL [22].

Statistical Analysis

The sample size for this study was based on CD4 percent as a correlate of antibody titer. For a 2-sided test with α=0.05, a sample size of 230 provided >80% power to observe a minimum 2.3-fold difference in positive antibody responses between children with a CD4 percent above and below 25%. Log-binomial regression with robust standard errors was used to compare characteristics of children who were measles IgG seropositive compared to those who were not. Stata version 11.1 was used for all analyses (StatCorp, College Station, TX).

RESULTS

Of the 272 children screened, 232 were enrolled. The most common reason for not enrolling was CD4% < 15, accounting for 11 (28%) of 40 screened but not enrolled. Children not enrolled did not differ from enrolled children by age, caregiver monthly rent, or caretaker occupation (data not shown).

Of 232 enrolled children, 228 (98%) had received one measles vaccine before one year of age, per Kenya immunization guidelines for the general population which recommend a single routine vaccination at 9 months. Measles vaccination was confirmed by presentation of a vaccination card for 106 (46%) caretakers, or by verbal report. Two caretakers (1%) reported no measles vaccination, and two reported that they were unsure whether their child was vaccinated. Male participants constituted 123 (53%) of the cohort, median CD4% was 32 (interquartile range [IQR] 27 – 38), and median age was 7.5 years (IQR 5.5 – 9.5 years). Of the 216 (93%) of children with recorded date of ART initiation, median time on ART was 3.4 years (IQR 1.8 – 4.9 years). Only 10 (10%) of 103 children with a vaccination card and recorded date of ART initiation were on treatment before the date of measles vaccination (Table 1).

Table 1. Predictors of a seropositive measles antibody titer among HIV-infected children on treatment.

	Total N[1]	Measles seropositive[2] n (%)	PR[3]	95% CI[4]	p-value
Age (years)					
<2	7	2 (29)	1.0	Ref.	
2 – 4.9	40	27 (68)	2.36	0.72 – 7.79	0.158
5+	185	96 (52)	1.81	0.56 – 5.92	0.322
CD4%					
< 25	44	14 (32)	1.0	Ref.	
≥ 25	188	111 (59)	1.86	1.18 – 2.91	**0.007**
Time on ART (years)					
<1	26	12 (46)	1.0	Ref.	
1 – 4.9	142	77 (54)	1.17	0.75 – 1.83	0.476
5+	48	30 (62)	1.35	0.84 – 2.17	0.207
Sex					
Female	109	61 (56)	1.0	Ref.	
Male	123	64 (52)	0.93	0.73 – 1.18	0.549
On ART before date of measles vaccination					
No	112	61 (54)	1.0	Ref.	
Yes	12	8 (67)	1.22	0.79 – 1.89	0.364

[1]N indicates total number of children in the category for whom data are available
[2]Measles seropositive refers to number of children who had a measles IgG antibody titer ≥ 200 mIU/mL
[3]PR indicates prevalence ratio; [4]CI confidence interval: [5]ART antiretroviral therapy

The majority, 190 (82%), of primary caregivers at the enrollment visit were the childrens' mothers. Primary caretakers' median age was 33 (IQR 30 – 38 years), and median years of completed school was 11 years (IQR 8 – 12). More than half of caregivers, 149 (64%), were married. Of the 199 (86%) renting their home, median monthly rent was 41 USD (IQR 24 – 59). Caretakers reported a median of 2 rooms (IQR 1 – 3) and 4 people (IQR 4 – 5) per household (data not shown).

At enrollment, 125 (53.9%) of 232 children were measles IgG seropositive. Median titer among those who were seropositive was 631 mIU/mL (IQR 385 – 1,902).

Children with CD4% ≥ 25 were 1.86 times as likely to be measles seropositive than children with a lower CD4% (RR 1.86, 95% CI 1.18 – 2.91). Children who were on ART prior to measles vaccination were 1.22 times as likely to be measles seropositive (RR 1.22, 95% CI 0.79 – 1.89), though this association was not statistically significant. There was no statistically significant association between age, gender, or time on ART and measles seropositivity (Table 1). No difference in measles seropositivity was observed between those whose caretaker presented a vaccination card compared to those whose caretaker verbally reported vaccination history.

DISCUSSION

In this Nairobi-based study, just over half of HIV-infected children on ART had seropositive measles-specific IgG antibody titers, and a CD4% ≥ 25 was identified as a predictor for measles seropositivity. Although the power to detect an association was limited, children who initiated ART prior to vaccination may have been more likely to be seropositive.

The duration of immunity following measles vaccination in healthy, non-HIV-infected children can persist for decades, especially in countries where measles remains endemic and

immune responses may be boosted by exposure to wild-type measles virus [23]. For example, a recent study in The Gambia found that 96% of HIV-uninfected children 8-9 years of age who were vaccinated once at 9 months of age had a seropositive measles antibody response [24]. However, similar results among HIV-uninfected children who received one measles vaccine dose have been reported in areas where measles is not endemic [25], suggesting re-exposure alone is not the sole determinant for persistent measles seroprotection.

The low level of measles seropositivity in this HIV-infected Kenyan cohort could be due to poor initial or unsustained immune response after primary measles vaccination. Our findings are comparable to two recent studies in HIV-infected children on treatment where 52% of HIV-infected American children and 42% of HIV-infected Thai children had were seropositive for measles antibody [26, 27]. These numbers are remarkably similar to our Kenyan cohort despite differences in treatment adherence, nutrition, type of measles vaccine, prevalence of measles disease, and living conditions, all variables that could affect the measles seropositivity in these three cohorts of HIV-infected children on treatment.

We also observed children with a CD4% ≥ 25 were more likely to have seropositive measles antibody titers. Interestingly, a CD4% ≥ 25 was also a predictor for measles seropositivity in the treated HIV-infected American children cohort referenced above [26]. However, this finding differs from what was found in a study of HIV-infected, treatment-naïve Kenyan children who had been previously vaccinated. In that study CD4% was not a predictor of measles seropositivity [3], and median levels of CD4% were 6.3% (IQR 3.0, 10.6), much lower than in our cohort of children on ART. It is possible that not only immune reconstitution, but the degree of reconstitution plays a role in the initial and sustained response to measles vaccination. A lower CD4% can be indicative of a low nadir CD4% or a poor response to ART, both of which could potentially inhibit measles antibody

seropositivity. Thus, by initiating ART early, per WHO recommendation, better responses to measles vaccination may be promoted by limiting HIV progression and preventing children from reaching a low nadir CD4%.

Though our findings are similar to recent studies, our estimate of measles seropositivity may be conservative. The gold standard for measles antibody detection is a plaque reduction neutralization (PRN) technique; a PRN titer ≥ 200 is considered the minimum protective correlate of immunity [22], which is equivalent to 200 mIU/mL. ELISA results are closely associated with PRN, though they lack sensitivity to detect measles antibody at low levels [28].

In summary, we observed a low prevalence of seropositive measles-specific IgG antibody titers despite previous measles vaccination in HIV-infected children on treatment. A number of measles outbreaks have occurred recently in Kenya where, in 2012, there were nearly 2500 confirmed cases of measles [29]. Considering these outbreaks and the low prevalence of measles seropositivity, a new vaccination strategy is needed for this population. HIV-infected children with a CD4% below 25 were observed to have particularly low prevalence of measles seropositivity, and may constitute a target group for re-immunization if resources are limited. Since all HIV-infected children on treatment may benefit by measles revaccination, the effectiveness and sustainability of vaccine response after re-immunization should also be explored.

Chapter 2: Nutritional status, HIV viral load, and sustained responses to measles revaccination in HIV-infected children on antiretroviral therapy in Kenya

INTRODUCTION

Despite recent advances in reducing overall measles incidence in Africa [1], a number of large and deadly measles outbreaks have occurred in countries with high adult and child HIV prevalence [2]. HIV infection reduces both initial and sustained responses to measles vaccination. Studies have shown that, in HIV-infected infants, a single measles vaccine at 9 months of age had an immediate protective response rate between 25% and 88% [3-5]. Further, even in those HIV-infected infants with an adequate initial response, fewer than 50% maintained protective antibody levels after one year [4, 5]. Vaccination after initiation of antiretroviral therapy may yield satisfactory response, however ART is often not initiated after vaccination at 9 months of age and thus HIV-infected children may receive measles vaccination when it is less likely to be effective.

Models predict an increase in prevalence of measles among HIV-infected and uninfected children as ART becomes more widely available, thus extending the life expectancy of HIV-infected children [6]. This would be a significant reversal of recent gains in reducing childhood morbidity and mortality due to measles. In areas where resources are not limited, an approach has been to provide ART and then revaccinate HIV-infected children once their immune systems have reconstituted [7, 8]. However, in a recent position paper on measles vaccination among HIV-infected children in resource-limited settings, the World Health Organization (WHO) stated that there was insufficient data about the effect of ART on measles antibody response due to a lack of studies on the subject, and concluded that there was not enough information to create a policy for revaccination against measles [9]. However, they do recommend a second opportunity for measles vaccination for all children through supplemental immunization activities or routine second dose. The scope and frequency of SIA vary by country, and many countries have yet to implement SIA [23].

We conducted a prospective study to determine the prevalence of measles seropositivity at one month and one year after administration of an additional measles vaccine in HIV-infected children on ART in Nairobi, Kenya. Additionally, we explored factors correlated with seroconversion at one year post revaccination among the subset of HIV-infected children who were seronegative at enrollment.

METHODS

Study participants

HIV-infected children ≥ 15 months to 12 years of age and their caregivers were recruited from the Comprehensive HIV Care Centre at Kenyatta National Hospital in Nairobi, Kenya, during routine HIV care visits from May 2011 to January 2013. Caregivers accompanying children to the clinic were provided an informational study pamphlet by our recruitment nurse. Those willing to be screened were asked to read and sign an informed consent document, approved by Kenyatta National Hospital and the University of Washington, if their child met eligibility criteria. Children above the age of 7 years were asked to assent to participation. Study participants and caregivers received additional education and counseling regarding measles and HIV/AIDS if requested. Those eligible but who chose not to enroll were given access to a measles vaccine through the clinic for low- to no-cost.

Eligibility criteria included confirmed HIV-1 infection confirmed by HIV-1/2 ELISA and/or PCR, and that the child was on ART at the time of enrollment, between the ages of 15 months and 12 years of age, and had a CD4% ≥15. Additionally, the caregiver had to be able and willing to provide adequate locator information and intend on remaining with the child in Nairobi for the next 2 years. Child exclusion criteria included a history of adverse reaction to measles vaccination, allergy to gelatin or neomycin, hospitalization within the last 2 weeks, blood or plasma transfusion within last 6 months, or receipt of immune globulin within the last 6 months prior to enrollment.

Procedures

During the enrollment visit, a questionnaire was administered to caregivers to obtain social and demographic data, current and past medical conditions, and relevant family history. The child received a physical exam, and immunization records, HIV diagnosis, and treatment information were obtained from the child's clinical chart. Caregivers were asked to present their child's immunization card, which documents all prior vaccinations, including measles. At the end of the enrollment visit, the child received a measles vaccination. Follow-up questionnaires and physical exams were administered at 1, 3, 6, 9, and 12 months after revaccination.

Laboratory assays

Blood draws for were obtained from children at enrollment, 1, 6, and 12 months after revaccination. Measles IgG was determined at enrollment, 1 month, and 12 months post revaccination. CD4% was determined at enrollment, 6 months, and 12 months post revaccination in accordance to standard clinical procedure that requires CD4% tracing every 6 months. HIV viral load was determined at enrollment and 12 months post revaccination

CD4% was determined in real time by the University of Nairobi Paediatrics Laboratory using internal quality controls (FACSCalibur, BD Biosciences, USA). To determine HIV viral load, plasma samples that were collected at enrollment and at 12 months post revaccination were frozen and shipped to Seattle, Washington, USA on dry ice and stored at −80°C until use. HIV-1 RNA levels were measured by the Gen-Probe HIV-1 viral load assay (Gen-Probe, San Diego, CA), which has been validated on the HIV subtypes prevalent in Kenya [30]. We considered a child to have virologic failure if their viral load was above 1,000 copies/mL, based on the current WHO definition of HIV viralogic failure in children [31].

Vitamin A deficiency is defined as a plasma retinol concentration of <0.7 umol/L, and moderate deficiency is defined as a plasma retinol concentration of 0.7 – 1.05 umol/L [32]. Circulating concentrations of plasma retinol are reduced by infection, in which case it may be a poor measure of vitamin A deficiency. Thurnham et al. developed a method to correct for infection-related low retinol concentrations by measuring two proteins that are a marker for inflammation: C-reactive protein (CRP), a measure of acute infection, and α-1-acid-glycoprotein (AGP), a measure of chronic infection, and adjusting measured retinol concentration [33]. In the present study, retinol-binding protein (RBP), which closely correlates to circulating plasma retinol levels [32], was measured, and corrections for inflammation were applied. Serum samples collected at enrollment were frozen and shipped to Wilstaett, Germany and RBP, CRP, and AGP were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) technique [34]. RBP was then adjusted for inflammation by increasing the concentration by 13% if CRP was elevated ≥5mg/L, by 11% if AGP was elevated ≥1g/L, and by 24% if both CRP and AGP were elevated [33].

Qualitative detection and quantitative determination of measles IgG antibodies were performed using an ELISA (Enzygnost, Germany), with internal quality controls performed for each run. Optical density (OD) values were determined and values >0.1 OD were converted into measles-specific IgG mIU/mL using manufacturer's calculations based on the First International World Health Organization (WHO) Reference Preparation Standards. Measles seropositivity was defined as a measles IgG titer ≥ 200 mIU/mL, a level that is considered protective against clinical measles [22].

Statistical analyses

Data from questionnaires were entered into a Microsoft Access Database, then checked, cleaned, and analysed using Stata (version 11.0). We defined response to measles

revaccination in two ways: seropositivity (a measles IgG titer ≥ 200 mIU/mL at any time point), and seroconversion (an increase in measles IgG antibody titer from undetectable levels at baseline to seropositive levels at 1 or 12 months post revaccination).

Height-for-age, weight-for-height, and weight-for-age z-scores were calculated using the WHO Child Growth Standards (WHO Anthro, version 3.2.2, January 2011) [35]. Stunting was defined as a height-for-age z-score of 2 or more standard deviations (SD) below the mean, wasting was defined as a weight-for height of 2 or more SD below the mean, and underweight was defined as weight-for-age 2 or more SD below the mean.

T-tests were used to determine differences between log transformed antibody titers at one month post revaccination by serostatus at 12 months post revaccination. In order to determine whether measles revaccination resulted in an increased prevalence of seropositivity at 12 months post revaccination, we estimated the risk of seropositivity at 12 months by log-binomial regression with robust error variance. The potential association of baseline characteristics and seropositivity at 12 months was univariately evaluated by log-binomial regression with robust error variance. Baseline characteristics assessed included age, sex, measles serostatus at enrollment, CD4%, HIV viral load, time on ART, WHO Stage, vitamin A deficiency, height-for-age z-score, and stunting. Graphical techniques were used to find biologically relevant cutoffs for continuous correlates. Maintenance of seropositivity is a different immunological mechanism than gaining seropositivity (seroconversion) between enrollment and one year post revaccination, thus those initially seronegative were separated to explore factors associated with seroconversion. We assessed the risk of seroconversion at 12 months post revaccination among those who were seronegative at enrollment by log-binomial regression with robust error variance. The same baseline characteristics as mentioned above were evaluated for an association with seroconversion at 12 months post revaccination. Characteristics that were statistically

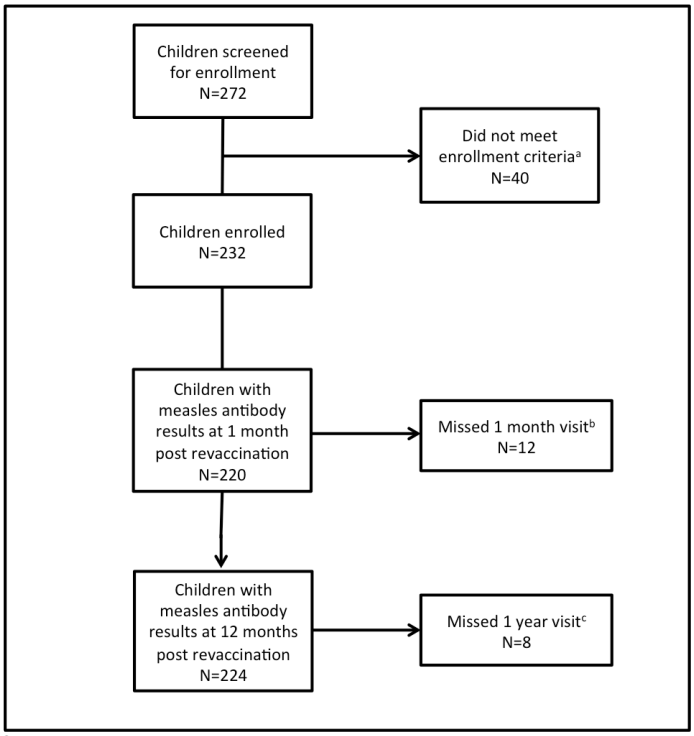
significantly associated with seroconversion at 12 months post revaccination were added to a multivariate model.

RESULTS

2.1 Cohort description

Of 272 children screened, 232 were enrolled and revaccinated. The most common reason for not enrolling was a CD4% < 15, accounting for 11 (28%) of 40 screened but not enrolled. Children not enrolled did not differ from enrolled children by age, caregiver monthly rent or caregiver occupation (data not shown). Of the enrolled children, 220 attended their one month visit and 224 attended their 12 month post revaccination visit (Figure 1).

Figure 1. Enrollment and follow-up of study participants



[a] Most common reason for not enrolling was a CD4%<15, accounting for 11 (28%) of 40 screened but not enrolled.
[b] Four (33%) of 12 missed a visit but returned to the study later, four left their study visit without a blood draw, and four were lost to follow-up due to caregivers who no longer wanted to participate in the study.
[c] Six (75%) of eight were lost to follow-up: Three had caregivers who did not want to participate any longer, two children relocated, and one child died of non-study related reasons. Two (25%) did not get a blood draw during their study visit.

Of 232 enrolled children, 228 (98%) had received at least 1 measles vaccine before 1 year of age, per Kenya immunization guidelines for the general population which recommend a single routine vaccination at 9 months. Measles vaccination was confirmed by presentation of a vaccination card for 106 (46%) caregivers or by verbal report. Two caregivers (1%) reported no previous measles vaccination, and 2 reported that they were unsure whether their child was vaccinated. Proportion of those measles seropositive at baseline did not differ significantly between children whose caregivers had a vaccination card and those who verbally reported immunization status. At enrollment, male participants constituted 123 (53%) of the cohort, median CD4% was 32 [interquartile range (IQR) 27–38], and median age was 7.5 years (IQR: 5.5–9.5 years). All children were on ART, and of the 216 (93%) of children with recorded date of ART initiation, median time on ART was 3.4 years (IQR: 1.8–4.9). At enrollment, 52 (22.5%) of 231 children had an HIV viral load ≥1,000 copies/mL, and 148 (70%) were recorded as having advanced stages of HIV (WHO Stage III or IV). (Table 2).

Table 2. Enrollment characteristics of HIV-infected children on antiretroviral therapy

	N or median	% or IQR[1]
Child Characteristics		
Demographic		
Age (years)	7.5	5.5 – 9.5
Male	123	53
Immunologic		
CD4%	32	27 – 38
CD4% ≥ 25	188	81
HIV viral load copies/mL	35	8 – 416
HIV viral load		
<50 copies/mL	141	61
50-999 copies/mL	38	16
≥1,000 copies/mL	52	23
Age at HIV diagnosis (years)	3.3	1.7 – 4.8
Age at ART[2] initiation	3.8	1.9 – 5.4
Years on ART at time of revaccination [3]	3.4	1.8 – 4.9
On ART at time of first measles vaccination[4]	10	10
WHO Stage[5]		
Not Advanced (I or II)	64	30
Advanced (III or IV)	148	70
Previous measles vaccinations[6]		
1	223	96
2	5	2
Nutritional		
Vitamin A status[7]		
Deficient (RBP <0.7)	7	3
Moderately deficient (RBP 0.7-1.05 umol/L)	52	23
Stunted	54	23
Underweight	11	5
Wasted	1	0.4

[1] Interquartile range; [2] Antiretroviral therapy (ART); [3] Out of 216 with record of ART history
[4] Out of 103 with vaccination card and ART history; [5] Out of 212 with clinical record history
[6] By verbal report or vaccination history card; [7] After correction for markers of inflammation;
[8] Retinol-binding protein (RBP)

Seven (3.1%) of 229 children had a vitamin A deficiency. Fifty-two (22.7%) of 229 children had levels of retinol binding protein, between 0.7 and 1.05 umol/L, that were considered moderately vitamin A deficient. Correction of RBP by CRP and AGP did not affect the number of children who were identified as vitamin A deficient or moderately deficient. There were 54 (23.2%) of children with stunting, as measured by a height-for-age ≤-2 (Table 1).

The majority, 190 (82%), of primary caregivers at the enrollment visit were the children's mothers. Primary caregivers' median age was 33 years (IQR: 30–38), and median years of completed school was 11 (IQR: 8–12). More than half of caregivers [149 (64%)], were married. Of the 199 (86%) renting their home, median monthly rent was 41 USD (IQR: 24–59). Caregivers reported a median of 2 rooms (IQR: 1–3) and 4 people (IQR: 4–5) per household (Table 3).

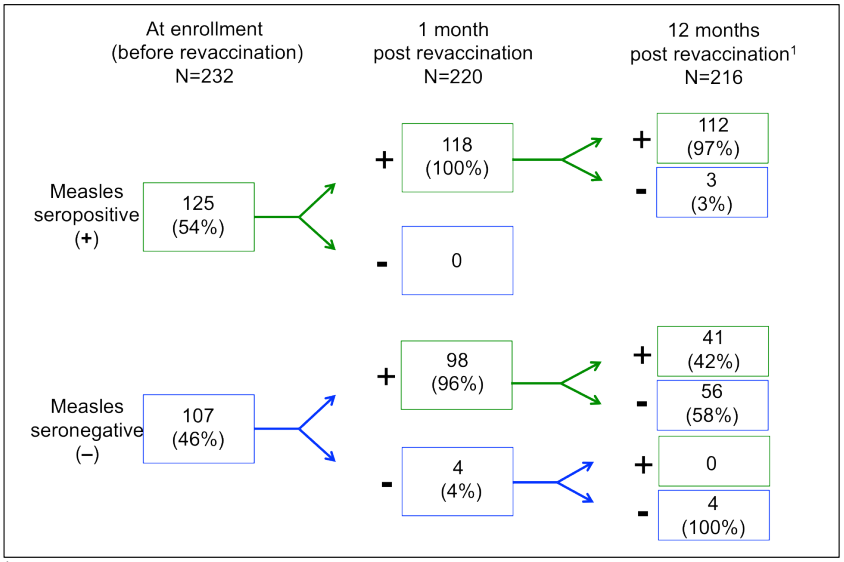
Table 3. Enrollment characteristics of caregivers of HIV-infected children on antiretroviral therapy

	N or median	% or IQR[1]
Caregiver Characteristics		
Mother is primary caregiver	190	82
Age	33	30 – 38
Years of completed school	11	8 – 12
Married	149	64
Renting home	199	86
Monthly rent if renting	41	24 – 59
Rooms per household	2	1 – 3
People per household	4	4 – 5

2.2 Serostatus at 1 and 12 months post revaccination among all participants

At enrollment, 125 (54%) of 232 participants were measles seropositive. A CD4% ≥ 25 was a correlate of seropositivity at enrollment, and has been previously described[36]. At one month post revaccination, 216 (98.1%) of 220 were seropositive, and at 12 months post revaccination, 158 (70.5%) of 224 were measles seropositive. Among those who were seropositive at enrollment, all remained seropositive at one month post revaccination, and 83 (96.5%) of 86 sustained their seropositivity at 12 months post revaccination. The three participants who were seropositive at enrollment and one month but lost seropositivity by 12 months post revaccination had measles-specific IgG antibody titers close to the seropositive threshold at enrollment and 12 months post revaccination. Among those who were seronegative at enrollment, 88 (95.7%) of 92 were seropositive at one month post revaccination, and 31 (34.0%) of 91 sustained seropositivity at 12 months post revaccination (Figure 2). No participants reported additional measles vaccinations through supplementary immunization activities between enrollment and 12 months post revaccination.

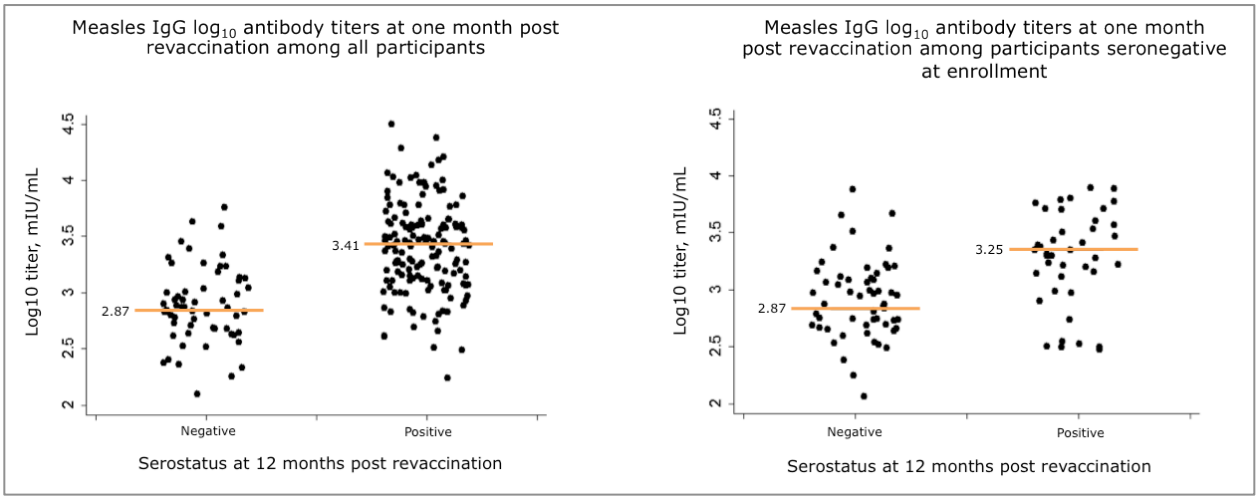
Figure 2. Measles serostatus of HIV-infected children on antiretroviral therapy at enrollment, one month, and 12 months post measles revaccination



[1] Eight participants who have measles serostatus data at 12 months are not included due to missing data from 1 month

Measles IgG log_{10} titers increased at one month post revaccination and decreased slightly by 12 months post revaccination. In the entire cohort, the log_{10} geometric mean concentration (GMC) of measles IgG antibody at one month post revaccination was higher in participants who sustained seropositivity at 12 months post revaccination (log_{10} GMC=3.41, Standard Deviation (SD) 0.35) compared to those who did not sustain seropositive levels of measles antibody at 12 months post revaccination (log_{10} GMC=2.87, SD 0.29, p<0.001). Among those who were seronegative at baseline, participants who seroconverted and sustained measles antibody titers at 12 months post revaccination also had stronger initial vaccine responses at one month post revaccination (log_{10} GMC=3.25, SD 0.34) compared to those who did not sustain seropositive levels of measles antibody (log_{10} GMC=2.87, SD 0.30, p<0.001)(Figure 3).

Figure 3. Measles IgG log_{10} antibody titers and log_{10} geometric mean at one month post revaccination among HIV-infected children on antiretrovirals, by serostatus at 12 months post revaccination



2.3 Factors associated with seropositivity at 12 months post revaccination among all participants

We assessed predictors of measles seropositivity of the 224 HIV-infected children on ART who attended a study visit at 12 months after revaccination. Children who were seropositive at enrollment were 2.47 times as likely to be seropositive at 12 months post revaccination compared to those who were seronegative at time of revaccination (risk ratio [RR] 2.47, 95% CI 1.94 – 3.15, p<0.001). Children with a CD4% ≥25 at enrollment were 1.39 times as likely to be seropositive at 12 months post revaccination (RR=1.39, 95% CI 1.04 – 1.87, p=0.026) compared to those with a CD4% <25, and children with an undetectable HIV viral load <50 copies/mL at time of revaccination were 1.42 times as likely to be seropositive compared to those with an HIV viral load ≥1000 copies/mL (RR=1.42, 95% CI 1.09 – 1.85, p=0.009).

While there was no association between being stunted at time of revaccination and seropositivity at 12 months post revaccination, height-for-age z-score was associated with seropositivity at 12 months post revaccination (RR=1.07, 95%CI 1.00 – 1.15, p=0.045). There were no statistically significant associations between the enrollment characteristics of age, gender, time on ART, WHO stage, or vitamin A status and seropositivity at 12 months post revaccination (Table 4).

Table 4. Predictors of a seropositive measles antibody titer at 12 months post revaccination among 224 HIV-infected children on antiretroviral therapy

Child characteristic at time of revaccination	Total N	Sero-positive n(%)	RR[1]	95% CI[2]	p-value
Demographic					
Age (years)					
<2	6	4 (67)	1.00	Referent	
2-4.9	38	28 (73)	1.11	0.61, 2.01	0.743
>5	180	126 (70)	1.10	0.59, 1.87	0.868
Sex					
Female	105	80 (76)	1.00	Referent	
Male	119	78 (66)	0.86	0.73, 1.02	0.081
Immunologic					
Serostatus at enrollment					
Seropositive	120	117 (98)	1.00	Referent	
Seronegative	104	41 (39)	2.47	1.94, 3.15	**<0.001**
CD4%					
<25%	43	23 (53)	1.00	Referent	
≥25%	181	135 (75)	1.39	1.04, 1.87	0.026
HIV viral load					
≥1,000 copies/mL	51	28 (12)	1.00	Referent	
50-999 copies/mL	36	23 (64)	1.16	0.82, 1.65	0.396
<50 copies/mL	137	107 (78)	1.42	1.09, 1.85	**0.009**
Time on ART[3] (years)					
<1	25	15 (60)	1.00	Referent	
1-4.9	136	97 (71)	1.19	0.85, 1.68	0.316
>5	47	37 (79)	1.31	0.92, 1.87	0.132
WHO Stage					
Early (I & II)	64	41 (64)	1.00	Referent	
Advanced (III & IV)	142	106 (75)	1.17	0.95, 1.43	0.149
Nutritional					
Vitamin A status					
Not deficient	164	114 (70)	1.00	Referent	
Moderately deficient	51	39 (76)	1.10	0.92, 1.32	0.308
Deficient	7	3 (43)	0.62	0.26, 1.46	0.272
Height-for-age z-score	224	158 (71)	1.07	1.00, 1.15	**0.045**
Stunting					
Not stunted	172	123 (72)	1.00	Referent	
Stunted	52	35 (67)	0.94	0.76, 1.16	0.576

[1] Relative Risk; [2] Confidence Interval; [3] Antiretroviral therapy

2.4 Factors associated with change in serostatus (seroconversion) at 12 months post revaccination among those seronegative at baseline

We investigated factors that were associated with seroconversion at 12 months post revaccination among the 104 participants who were seronegative at enrollment. Children with an HIV viral load <50 copies/ml were twice as likely to seroconvert and maintain measles antibody seropositivity compared to children who had an HIV viral load ≥1000 copies/mL (RR=2.04, 95% CI 1.01 – 4.10, p=0.047). A larger height-for-age z-score at enrollment was also associated with seroconversion at 12 months post revaccination (RR=1.24, 95% CI 1.04 – 1.48, p=0.016). CD4%, while statistically significant when assessing seropositivity in the entire cohort, was not significantly associated with seroconversion at 12 months post revaccination among those who were seronegative at time of revaccination. Moderate vitamin A deficiency was not significantly associated with seroconversion and 12 months post revaccination, and of the 4 participants who had vitamin A deficiency, zero of them seroconverted at 12 months post revaccination. Stunting, WHO stage, time on ART, age, and gender were not statistically significantly associated with seroconversion at 12 months post revaccination. An HIV viral load <50 copies/ml and larger height-for-age remained significant in a multivariate model that included both variables (Table 5).

Table 5. Predictors of a change in measles antibody serostatus at 12 months post revaccination among 104 HIV-infected children on antiretroviral therapy who were seronegative at enrollment

Child characteristic at time of revaccination	Total N	Sero-converted n (%)	RR[1]	95% CI[2]	p-value	Adjusted RR[4]	95% CI	p-value
Demographic								
Age (years)								
<2	4	2 (50)	1.00	Referent		--	--	--
2-4.9	12	4 (33)	0.67	0.19, 2.38	0.532	--	--	--
5+	88	35 (40)	0.80	0.29, 2.20	0.660	--	--	--
Sex								
Female	46	21 (46)	1.00	Referent		--	--	--
Male	58	20 (34)	0.76	0.47, 1.22	0.249	--	--	--
Immunologic								
CD4%								
<25%	29	9 (31)	1.00	Referent		--	--	--
≥25%	74	32 (43)	1.37	0.75, 2.52	0.303	--	--	--
HIV Viral Load								
≥1,000 copies/mL	29	7 (24)	1.00	Referent				
50-999 copies/mL	18	6 (33)	1.38	0.55, 3.47	0.493	1.49	0.56, 3.92	0.421
<50 copies/mL	57	28 (49)	2.04	1.01, 4.10	**0.047**	2.05	1.02, 4.12	**0.043**
Time on ART[3] (years)								
<1	13	4 (31)	1.00	Referent				
1-4.9	63	26 (41)	1.34	0.56, 3.21	0.509	--	--	--
5+	18	8 (50)	1.44	0.55, 3.81	0.458	--	--	--
WHO Stage								
Early (I & II)	36	14 (39)	1.00	Referent				
Advanced (III & IV)	59	25 (42)	1.09	0.66, 1.81	0.741	--	--	--
Nutritional								
Vitamin A status								
Not deficient	84	37 (44)	1.00	Referent				
Moderately deficient	16	4 (25)	0.57	0.23, 1.38	0.211	0.75	0.28, 1.99	0.561
Deficient	4	0 (0)	0.00	0, 0	**0.000**	0.00	0.00, 0.00	**0.000**
Height-for-age z-score	104	41 (39)	1.24	1.04, 1.48	**0.016**	1.23	1.04, 1.47	**0.018**
Stunting								
Not stunted	83	36 (43)	1.00	Ref.				
Stunted	21	5 (24)	0.55	0.24, 1.23	0.145	--	--	--

[1] Relative Risk; [2] Confidence Interval; [3] Antiretroviral therapy; [4] Adjusted for variables that were significant in univariate analysis: HIV viral load, vitamin A status, height-for-age z-score

DISCUSSION

In this prospective study on the effectiveness of measles revaccination in HIV-infected Kenyan children on ART, we found that 70.5% of children had seropositive levels of measles antibody at 12 months after revaccination compared to 54% prior to revaccination. This is the largest study to describe the results of measles revaccination in HIV infected children on ART in Sub-Saharan Africa, a region of high HIV and measles burden.

Measles seropositivity in overall cohort at 12 months post revaccination

A number of factors were associated with measles seropositivity in the overall cohort at 12 months post revaccination. Measles seropositivity at time of revaccination was the greatest predictor of seropositivity at 12 months post revaccination, though HIV viral load, CD4%, and height-for-age z-score were also associated with maintaining seropositivity or seroconverting in the overall cohort. Among those who were seronegative at enrollment, HIV viral load and height-for-age z-score were significant predictors of seroconversion at 12 months post revaccination. It has been shown that HIV infection affects the quantity and quality of antibody response to measles vaccination [37-39], and that initiation of ART alone is not sufficient to restore measles antibody response after vaccination [3]. Children in this Kenyan cohort did not respond to measles revaccination as well as other reported cohorts. A study in the United States found that approximately half of HIV-infected children lacked protective levels of measles antibody despite more than 80% receiving two doses of the measles, mumps, and rubella (MMR) vaccine [26]. Two months after revaccination, seropositive prevalence increased to 89%, and then fell to 80% by 1.5 years post revaccination [26]. In Thailand, Arpibul found 42% of HIV-infected children on ART had protective levels of measles antibody, which increased to 90% one month after revaccination and fell to 85% by 3 years after revaccination [7, 27, 40]. One reason for the differences may be HIV suppression, as 97% of children in the Thailand cohort were HIV

suppressed [40]. In the US cohort, approximately 60% of children had low or undetectable levels of HIV virus suppression, which is similar to this Kenyan cohort.

Change in measles serostatus in subset of children who were seronegative at enrollment

In the subset of children who were measles seronegative at enrollment, a change in serostatus (seroconversion) and a sustained response to measles revaccination was greatest in children with an undetectable level of HIV viral load and a larger height-for-age z-score. A sustained response was not related to CD4%. Other studies of measles revaccination in HIV-infected children on ART found that the response was more related to a state of suppressed HIV viral load than to CD4% [8, 26]. Amongst those seronegative at enrollment, 96% of children initially responded to measles revaccination at one month, but only 36% sustained seropositive levels of measles antibody by 12 months post revaccination. This suggests that the ability to sustain an immune response to measles vaccination may be hindered by high levels of HIV virus, which is associated with turnover and depletion of B and T cells needed for memory [41]. Though all of the children in this study were on ART, nearly 40% had detectable levels of HIV virus, and more than 20% had virologic failure, as defined by an HIV viral load greater or equal to 1000 copies/ml. Full immune reconstitution as measured by HIV viral load, not CD4%, may be necessary before measles revaccination is successful.

In the subgroup of children with an undetectable HIV viral load, nearly 80% of children who were seronegative at enrollment were able to seroconvert and sustain measles specific antibodies at 12 months post revaccination. Provision of a second or third vaccine to this subgroup of HIV-infected children was still less effective than a single measles dose is in uninfected children. In uninfected children, a single measles vaccine can confer decades-long protective levels of measles antibodies in 85 - 95% of children [23-25]. Other

mechanisms of sustained immune response, including cell mediated immunity, must be explored to optimize measles revaccination efforts in HIV-infected children.

Vitamin A deficiency may be associated with sustaining an immune response to measles vaccination, but the number of participants with vitamin A deficiency were too small to robustly estimate its effect. It is well established that vitamin A plays an influential role in the regulation of immune responses [13-17]. Vitamin A supplementation, an inexpensive and easily implemented intervention, may improve measles antibody response [18, 19], though there has been conflicting evidence about the degree of the benefit [20, 21]. These previous studies were in HIV-uninfected child cohorts, where measles seroconversion rates were high, with small differences in seroconversion between supplemented and unsupplemented groups. In HIV-infected children, where seroconversion rate and sustained antibody response are much lower, nutrition deficiencies may play a greater role.

Height-for-age z-score, a measurement of chronic undernutrition, was associated with the ability to mount and sustain an immune response to measles revaccination. Other studies have also found that measles immunization response rates in HIV-infected children may be reduced by malnutrition [10]. In a study of measles vaccination in HIV-infected children not on ART in Zambia, children who were stunted had lower antibody levels after measles vaccination compared to those who were not stunted, though stunting was not associated with measles seropositivity [4]. Undernutrition during childhood growth and development can impair development of the immune system [11]. Chronic undernutrition affects both acquired immunity and innate host defense mechanisms by impairing thymic development, T cell differentiation, T cell expansion, T cell memory, and macrophage activation [12]. Therefore, children who suffer from chronic malnourishment may have immune mechanisms that are unable to support sustained measles antibody responses. Further investigation of the immune mechanisms related to successful revaccination should be explored.

Limitations

Use of an ELISA to determine protection against clinical measles has its limitations. The current gold standard for measles antibody detection is a plaque reduction neutralization (PRN) technique; a PRN titer ≥120 is considered the minimum protective antibody titer [22], which is equivalent to 120mIU/mL. While we did not use a PRN technique, ELISA results are closely associated with PRN, though they lack sensitivity to detect measles antibody at low levels [28]. Thus, ours may be a conservative estimate of percentage protected against measles. The smallest antibody concentration detectable using our commercially available ELISA is approximately 200 mIU/mL, which is a higher titer than what is considered minimally protective against clinical measles. Another limitation is the use of retinol-binding protein as a surrogate for vitamin A. Though direct measurement of vitamin A plasma retinol is possible using HPLC, clinical and lab conditions were such that samples could not be processed and stored in a way to reduce degradation of vitamin A plasma retinol by light. However, retinol binding protein is a sensitive and specific surrogate measure of plasma retinol [34, 42], even in HIV-infected individuals [43], and is often used as a reliable surrogate.

We cannot compare how many children who were seropositive at time of revaccination would stay seropositive in the absence of revaccination since the study did not include a group of HIV-infected children who were not revaccinated. The measles antibody status of children prior to enrollment in the study was unknown, and we did not want to withhold a potentially beneficial intervention from participants in this particularly susceptible group, so measles vaccine was provided to all participants. However, determination of pre-and post-vaccination measles IgG antibody levels in this cohort is a valid assessment of the potential benefit of measles revaccination in a population of HIV-infected children who regularly attend a clinic for prescription refills and HIV disease monitoring. Since measles antibody

determination is not a standard of care, the study also reflects a real-life scenario of measles revaccination in a population without routine testing for measles antibody levels.

This study provides evidence that measles revaccination is successful in HIV-infected children who are immune reconstituted on ART. The current policy in Kenya is to provide one measles vaccine as part of a routine immunization schedule with a second opportunity through supplementary immunization activities (SIA). In Kenya, there have been five SIA campaigns between 2005 and 2012 in response to measles outbreaks [44]. These SIA campaigns typically focus on children under five, and provide measles vaccines to children in schools. Since one dose of measles vaccine is 85-95% effective in healthy children [45] yet only 42-54% effective in HIV-infected children [26, 27, 36], it is especially important to reach children with HIV, especially those who are over the age of 5, in order to achieve measles elimination goals. HIV care clinics may be a good place for targeted campaigns to facilitate additional measles vaccination opportunities for this susceptible population. Children with HIV who are on ART attend clinic every few months to get a prescription refill and for disease monitoring, so a clinic-based delivery system could more directly access this susceptible group in a manner that minimizes cost in terms of human and physical resources.

Conclusion

Measles revaccination in immune reconstituted HIV-infected children on ART may be useful in improving and maintaining population wide levels of protective levels of measles antibody in areas of high HIV prevalence. HIV-infected children on ART are seen regularly for disease monitoring and provision of ART and constitute a group that may be easy to reach for revaccination with minimal cost and human resource expenditure. Nutritional food supplementation programs need to be strengthened and scaled up to ensure long term nutritional provisions for HIV-infected children who are taking ART, as nutrition may affect

vaccination success. Finally, this study reinforces the importance of HIV viral load suppression with ART to achieve maximum effectiveness of measles revaccination.

Summary & Next Steps

This study provides evidence that measles revaccination can increase the prevalence of sustained measles antibody responses in a population of HIV-infected children on ART. In a cohort of Kenyan HIV-infected children on ART with low levels of baseline measles antibody, measles revaccination was most successful in those who had suppressed levels of HIV viral load and higher height-for-age z-scores. Both immune reconstitution and nutritional status may be important to the measles immunization response in HIV-infected children.

There are three major next steps. First, further investigation of cellular-mediated immunity and nutritional factors that affect the immune response to vaccination against measles or other pathogens in HIV-infected children on ART is necessary. Though HIV viral load and nutritional status were important factors in a sustained measles revaccination response, the rates of sustained responses were still lower than a single dose of measles vaccine in HIV-uninfected children. Vitamin A deficiency seemed to hinder a sustained immune response, but the number of children with vitamin A deficiency was so small that this observation deserves more study.

Second, this cohort or others should be followed for a longer period of time in order to determine if the seropositive levels of measles antibody observed in this study are robust. More information about the rate of decline of protective levels of measles antibody would be useful to inform measles vaccination policy regarding how often a the population of HIV-infected children on ART may need to get additional measles vaccinations. This type of follow up could be done in a programmatic setting, since measles vaccine is safe for HIV-infected children with a CD4%≥15. Implementation science studies could go further and look at more than one revaccination if measles antibody titers wane. This could help inform measles vaccination policy specific to HIV-infected children on ART.

Third, data from this study should be used to generate parameters for measles transmission models that take into account the growing population of HIV-infected children. A measles transmission model, either Kenya or region-specific, can help assess the burden of measles cases that may be attributable to HIV-infection, and determine the potential effects of revaccination on future measles incidence. These data would be useful for policy makers in assessing disease burden and program changes that are needed to eliminate measles. The Kenya Measles Technical Advisory Group is particularly interested in a model of measles transmission that takes into account HIV-infected children and their vaccine effectiveness and waning immunity. Previous papers that model measles transmission have either not taken into account the effect of an immunized but unprotected HIV-infected population [46], or have considered measles transmission in an untreated HIV-infected child population with very high mortality [6]. It is important to consider the effect of the growing population of HIV-infected children on HAART on measles transmission, and how much it could cost to effectively vaccinate this vulnerable population. The cost-effectiveness of immunization program scenarios should also be established to provide information about the financial burden of potentially introducing a second routine immunization for HIV-infected children.

This study provides much needed data on effectively vaccinating HIV-infected children on ART against measles, and moves the field closer to establishing guidelines to protect this vulnerable population. In order to accomplish these next steps as outlined above, it is necessary to engage Kenya Ministry of Health and WHO officials. We are planning on sharing these data with Kenya Ministry of Health and the Kenya Measles Technical Advisory Group in the upcoming months, and will reach out to WHO officials in Nairobi, as well. Dissemination of the results will add to the national and regional discussion of how to best eliminate measles in the context of a growing population of HIV-infected children. These study results are an important contribution to that discussion.

References

1. Otten, M., et al., *Public-health impact of accelerated measles control in the WHO African Region 2000-03.* Lancet, 2005. 366(9488): p. 832-9.

2. Nilsson, A. and F. Chiodi, *Measles outbreak in Africa--is there a link to the HIV-1 epidemic?* PLoS Pathog, 2011. 7(2): p. e1001241.

3. Farquhar, C., et al., *Immune responses to measles and tetanus vaccines among Kenyan human immunodeficiency virus type 1 (HIV-1)-infected children pre- and post-highly active antiretroviral therapy and revaccination.* Pediatr Infect Dis J, 2009. 28(4): p. 295-9.

4. Moss, W.J., et al., *Immunogenicity of standard-titer measles vaccine in HIV-1-infected and uninfected Zambian children: an observational study.* J Infect Dis, 2007. 196(3): p. 347-55.

5. Thaithumyanon, P., et al., *Immune responses to measles immunization and the impacts on HIV-infected children.* Southeast Asian J Trop Med Public Health, 2000. 31(4): p. 658-62.

6. Scott, S., et al., *Predicted impact of the HIV-1 epidemic on measles in developing countries: results from a dynamic age-structured model.* Int J Epidemiol, 2008. 37(2): p. 356-67.

7. Aurpibul, L., et al., *Response to measles, mumps, and rubella revaccination in HIV-infected children with immune recovery after highly active antiretroviral therapy.* Clin Infect Dis, 2007. 45(5): p. 637-42.

8. Berkelhamer, S., et al., *Effect of highly active antiretroviral therapy on the serological response to additional measles vaccinations in human immunodeficiency virus-infected children.* Clin Infect Dis, 2001. 32(7): p. 1090-4.

9. *WHO position on measles vaccines.* Vaccine, 2009. 27(52): p. 7219-21.

10. Waibale, P., et al., *The effect of human immunodeficiency virus-1 infection and stunting on measles immunoglobulin-G levels in children vaccinated against measles in Uganda.* Int J Epidemiol, 1999. 28(2): p. 341-6.

11. Cunningham-Rundles, S., D.F. McNeeley, and A. Moon, *Mechanisms of nutrient modulation of the immune response.* J Allergy Clin Immunol, 2005. 115(6): p. 1119-28; quiz 1129.

12. Schaible, U.E. and S.H. Kaufmann, *Malnutrition and infection: complex mechanisms and global impacts.* PLoS Med, 2007. 4(5): p. e115.

13. Chandra, R.K., *Numerical and functional deficiency in T helper cells in protein energy malnutrition.* Clin Exp Immunol, 1983. 51(1): p. 126-32.

14. Chandra, R.K., *Rosette-forming T lymphocytes and cell-mediated immunity in malnutrition.* Br Med J, 1974. 3(5931): p. 608-9.

15. Ambrus, J.L., Sr. and J.L. Ambrus, Jr., *Nutrition and infectious diseases in developing countries and problems of acquired immunodeficiency syndrome.* Exp Biol Med (Maywood), 2004. 229(6): p. 464-72.

16. Semba, R.D., *Vitamin A, immunity, and infection.* Clin Infect Dis, 1994. 19(3): p. 489-99.

17. Kim, C.H., *Regulation of FoxP3 regulatory T cells and Th17 cells by retinoids.* Clin Dev Immunol, 2008. 2008: p. 416910.

18. Benn, C.S., et al., *Effect of vitamin A supplementation on measles-specific antibody levels in Guinea-Bissau.* Lancet, 2002. 359(9314): p. 1313-4.

19. Benn, C.S., et al., *Randomised trial of effect of vitamin A supplementation on antibody response to measles vaccine in Guinea-Bissau, west Africa.* Lancet, 1997. 350(9071): p. 101-5.

20. Bahl, R., et al., *Vitamin A administered with measles vaccine to nine-month-old infants does not reduce vaccine immunogenicity.* J Nutr, 1999. 129(8): p. 1569-73.

21. Semba, R.D., et al., *Effect of vitamin A supplementation on measles vaccination in nine-month-old infants.* Public Health, 1997. 111(4): p. 245-7.

22. Chen, R.T., et al., *Measles antibody: reevaluation of protective titers.* J Infect Dis, 1990. 162(5): p. 1036-42.

23. WHO. *Measles Vaccines: World Health Organization Position Paper*. Weekly epidemiological record 2009 [cited 84; pg. 349-360]. Available from: http://www.who.int/wer/2009/wer8435/en/index.html.

24. Viviani, S., et al., *EPI vaccines-induced antibody prevalence in 8-9 year-olds in The Gambia.* Trop Med Int Health, 2004. 9(10): p. 1044-9.

25. Demicheli, V., et al., *Vaccines for measles, mumps and rubella in children.* Cochrane Database Syst Rev, 2012. 2: p. CD004407.

26. Abzug, M.J., et al., *Immunogenicity, immunologic memory, and safety following measles revaccination in HIV-infected children receiving highly active antiretroviral therapy.* J Infect Dis, 2012. 206(4): p. 512-22.

27. Aurpibul, L., et al., *Prevalence of protective antibody against measles in HIV-infected children with immune recovery after highly active antiretroviral therapy.* HIV Med, 2006. 7(7): p. 467-70.

28. Ratnam, S., et al., *Comparison of commercial enzyme immunoassay kits with plaque reduction neutralization test for detection of measles virus antibody.* J Clin Microbiol, 1995. 33(4): p. 811-5.

29. WHO. *Reported measles cases and incidence rates by WHO Member States 2013*. 2013 05Mar2013]; Available from: http://www.who.int/immunization_monitoring/diseases/measlesreportedcasesbycountry.pdf.

30. Emery, S., et al., *Evaluation of performance of the Gen-Probe human immunodeficiency virus type 1 viral load assay using primary subtype A, C, and D isolates from Kenya.* J Clin Microbiol, 2000. 38(7): p. 2688-95.

31. WHO, *Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access*, 2010.

32. World Health Organization, *Indicators for assessing Vitamin A Deficiency and their application in monitoring and evaluating intervention programmes*, 1996.

33. Thurnham, D.I., et al., *Effects of subclinical infection on plasma retinol concentrations and assessment of prevalence of vitamin A deficiency: meta-analysis.* Lancet, 2003. 362(9401): p. 2052-8.

34. Erhardt, J.G., et al., *Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique.* J Nutr, 2004. 134(11): p. 3127-32.

35. World Health Organization, *WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development.* , 2006: Geneva. p. 312 pages.

36. Newman, L.P., et al., *Measles Seropositivity in HIV-Infected Kenyan Children on Antiretroviral Therapy.* Pediatr Infect Dis J, 2014.

37. Nair, N., et al., *HIV-1 infection in Zambian children impairs the development and avidity maturation of measles virus-specific immunoglobulin G after vaccination and infection.* J Infect Dis, 2009. 200(7): p. 1031-8.

38. Fowlkes, A., et al., *Persistence of vaccine-induced measles antibody beyond age 12 months: a comparison of response to one and two doses of Edmonston-Zagreb*

measles vaccine among HIV-infected and uninfected children in Malawi. J Infect Dis, 2011. 204 Suppl 1: p. S149-57.

39. Helfand, R.F., et al., *Evaluation of the immune response to a 2-dose measles vaccination schedule administered at 6 and 9 months of age to HIV-infected and HIV-uninfected children in Malawi.* J Infect Dis, 2008. 198(10): p. 1457-65.

40. Aurpibul, L., et al., *Persistence of measles, mumps, and rubella protective antibodies 3 years after revaccination in HIV-infected children receiving antiretroviral therapy.* Clin Infect Dis, 2010. 50(10): p. 1415-8.

41. Rainwater-Lovett, K. and W.J. Moss, *Immunologic basis for revaccination of HIV-infected children receiving HAART.* Future Virol, 2011. 6(1): p. 59-71.

42. Engle-Stone, R., et al., *Plasma retinol-binding protein predicts plasma retinol concentration in both infected and uninfected Cameroonian women and children.* J Nutr, 2011. 141(12): p. 2233-41.

43. Baeten, J.M., et al., *Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1 infection, protein malnutrition, and the acute phase response.* Am J Clin Nutr, 2004. 79(2): p. 218-25.

44. World Health Organization. 2014 [cited 2014 July 06]; Available from: http://www.who.int/immunization/monitoring_surveillance/data/en/.

45. Levin, A., et al., *Global eradication of measles: an epidemiologic and economic evaluation.* J Infect Dis, 2011. 204 Suppl 1: p. S98-106.

46. Bauch, C.T., E. Szusz, and L.P. Garrison, *Scheduling of measles vaccination in low-income countries: projections of a dynamic model.* Vaccine, 2009. 27(31): p. 4090-8.