Out of the

Division of Infectious Diseases and Tropical Medicine, University Hospital, LMU Munich, Germany

&

German Centre for Infection Research (DZIF), partner site Munich, Germany

Exploring the relationship between nutritional status and immunogenicity of routine childhood vaccines

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Dr Olubukola T. Idoko

born in

Zaria, Nigeria

submitted on

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Supervisors LMU:	Title, first name, last name
Habilitated Supervisor	Priv. Doz. Dr. Christof Geldmacher
Direct Supervisor	Priv. Doz. Dr. Elmar Saathoff
Supervisor External:	
Local Supervisor	Prof Beate Kampmann
Reviewing Experts:	
1st Reviewer	Priv. Doz. Dr. Christof Geldmacher
2nd Reviewer	Priv. Doz. Dr. Elmar Saathoff
Dean:	Prof. Dr. med. dent. Reinhard Hickel
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Nutritional status, immunogenicity, childhood, vaccine, yellow fever, measles, iron

Abstract

Background

Infection and malnutrition remain significant causes of under-five morbidity and mortality in sub-Saharan Africa with a cyclical relationship between infection and malnutrition. Malnourished children are more prone to infection and would benefit from vaccination which is the most cost-effective tool available against infectious disease. However due to nutrition-induced immune suppression the malnourished child may not respond as well to vaccination.

Methods

We explored the relationship between undernutrition and immune responses measured by antibody titres/IgG concentrations following one dose of yellow fever and measles vaccines in two cohorts of African children. Based on findings from our initial analysis we also assessed the longevity of responses to yellow fever vaccine in a third cohort of African children. World Health Organization z-scores for weight for age, weight for height and height for age were used to assess nutritional status. Vaccine responses to measles and yellow fever were measured by standard ELISA and sero/micro neutralization respectively. Linear and logistic regression models were built to assess the relationships between the covariates and vaccine responses. In addition, we explored correlation of a known immune modulator which is also a nutritional factor – iron on vaccine response.

Results

There was significantly higher seroconversion following yellow fever vaccination in Mali compared to Ghana (91.0 vs 63.5% ($p < 0.001$), and a trend to better seroconversion at higher height for age scores. Females attained significantly higher post vaccination antibody concentrations than males ($p<0.001$) following measles vaccination. Five to six years post yellow fever vaccination within routine programmes one quarter of the children no longer had protective titers. Iron metabolism is down regulated in the first week of neonatal life.

Conclusions

Chronic malnutrition may negatively impact responses to yellow fever vaccines. Five to six years post routine vaccination over 25% of children were no longer protected (defined by antibody concentrations) from yellow fever suggesting vulnerability to disease. There may be scope for considering differing vaccines doses for boys and girls for measles vaccine.

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i) Abbreviations

HIV	Human immunodeficiency virus
BCG	Bacille Calmette Guerin
EPI	Expanded Programme on Immunization
WHO	World Health Organization
MVP	Meningitis Vaccine Project
MenAfri-Vac/PsA-TT	Serogroup A meningococcal conjugate vaccine conjugated to tetanus toxoid
PFU	Plaque-forming units
RKI	Robert Koch Institute
$TCID_{50}$	Tissue culture infectious disease dose, 50%
UNISI	University of Siena in Italy
IPV	Inactivated polio vaccine
Ab	Antibody
PATH	Program for Appropriate Technology in Health
LMIC	Low- and middle-income countries
Hib-TT	Haemophilus influenza type b vaccine conjugated to tetanus toxoid
PsACWY	Polysaccharide tetravalent vaccine containing serogroup A, C, W and Y meningococcal strains
OD	Optical density
MMR	Measles, mumps rubella vaccine
UN IGME	United Nations Inter-Agency Group for Child Mortality Estimation

1. Introduction

1.1 Worldwide under five mortality

In 2017 alone, an estimated 5.5 million children under the age of 5 years died around the world [1, 2]. This accounts for 15,000 children every day [1, 3]. While these deaths represent less than half the number of deaths in 1990 [1] these mortality figures remain unacceptably high, especially given that the mortality is mainly due to preventable causes [1]. Half of the deaths occurred in sub-Saharan Africa with a median under 5 mortality of 2.6 million [2] and an under five mortality rate of 76 deaths per 1000 live births in 2017 [2]. One in 13 children in this region do not reach their 5th birthday with wide regional differences in these figures[2].

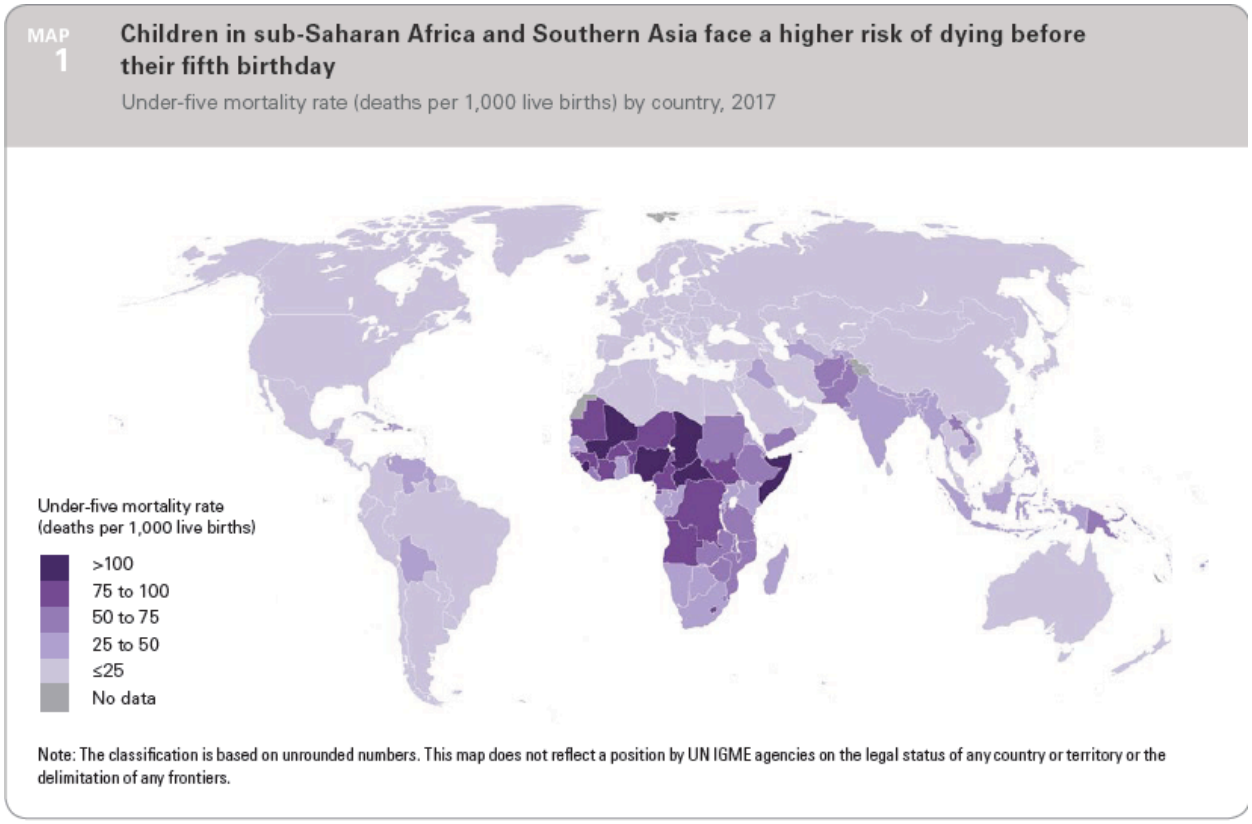


Figure 1.1: Global distribution of under 5 mortality

Source: United Nations Inter-Agency Group for Child Mortality Estimation (UN IGME) 2018

1.1.1 Infection as a leading cause of under-five mortality

The leading causes of under five deaths in 2017 were preterm birth complications, pneumonia, birth asphyxia, diarrhea and malaria [4]. Infectious disease thus remains a leading cause of death in children globally contributing to millions of the under five deaths [5-8]. The highest burden of infectious disease is also in sub-Saharan Africa with significant morbidity and mortality [5, 7].

1.1.2 Vaccines as a tool to combat morbidity and mortality

Vaccines are established as the most effective preventive intervention against infectious disease , [9-11] and it is estimated that vaccines have contributed to saving over 20 million lives and $350 billion between 2001 and 2017 in 73 low and middle income countries [10]. It is documented that vaccines save 2- 3 million lives each year worldwide [12]. Vaccines have not only contributed to disease prevention and thus prevention of mortality, but in 1976 led to the eradication of smallpox [13-15]. With the current drive to eradicate polio largely driven by vaccination, prospects for eradication are also in sight [16-18]. Rinderpest in animals has also been eradicated using vaccine interventions [19].

These extremely useful interventions however do not perform equally well in all settings and reactogenicity and immunogenicity have been reported to vary, depending on vaccine types and geographical regions [20-22]. Oral vaccines including oral polio vaccine and rotavirus vaccine are well documented to elicit poorer immune responses in resource poor settings [23, 24]. Unpublished data from my previous work also revealed that the reactogenicity to the 13-valent pneumococcal conjugate vaccine varied widely from region to region even where identical delivery mechanisms were utilized. Studies exploring some of the factors that mediate these differences in vaccine responses from region to region may contribute data that ensures optimal use of available interventions and provide a basis for modifying interventions to suit populations or modifying the host to enhance response to the intervention. This may include the need to modify vaccination schedules, provide supplements along with immunization programmes or modify environmental conditions in a drive to derive the best strategies for individuals and populations alike.

1.2 Malnutrition and infection and immunity

In addition to infectious diseases, malnutrition contributes significantly to nearly half of the annual deaths in the under-five age group [1, 25]. In 2017, nutrition related factors contributed to 45% of the deaths in children under 5 years of age [4, 26]. Closely linked to the morbidity and mortality due to malnutrition is the greater susceptibility of malnourished children to infection. Malnourished children have the highest risk of death from preventable infectious diseases such as measles, diarrhoea, malaria and pneumonia, especially in the context of acute undernutrition [3, 25, 27, 28]. In 2017, infectious diseases contributed to 41% of the deaths in malnourished children [25, 29].

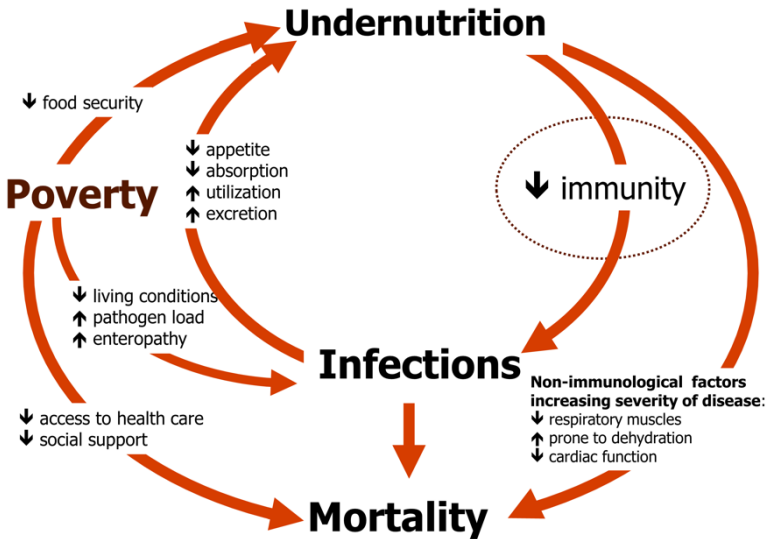


Figure 1.2: Conceptual framework on the relationship between malnutrition, infections and poverty.
Source: Rytter MJH et al. doi:10.1371/journal.pone.0105017.g001

1.2.1 Malnutrition and vaccine responses

As vaccines are proven to be a cost-effective intervention against infectious disease morbidity and mortality in general, vaccinating malnourished children is likely to be of particularly high benefit given the unique predisposition of these children to infectious disease. However, the vaccines might not work equally well in the malnourished for the same reason they are predisposed to infection – malnutrition-associated immune suppression [27]. The impaired ability to overcome infectious diseases is thought to be due in part to deficiencies in the innate, and adaptive arms of immunity documented in these children [30]. Undernutrition is also associated with impaired gut barrier function, implicated in the poorer responses to oral vaccines [23, 31]. Also documented is reduction in exocrine secretion of protective substances and decreased concentrations of plasma complement [31]. The thymus and other lymphatic tissue undergo atrophy and delayed type hypersensitivity reactions are less pronounced [31].

These factors might therefore affect not only responses to infection but also to vaccination. Vaccination guidelines for immunosuppressed children with advanced human immunodeficiency virus (HIV) infection and primary immune deficiencies recommend the avoidance of live vaccines which can cause severe or fatal reactions due to uncontrolled replication of the vaccine virus or bacterium [32, 33]. In addition, the local and regional reactions which follow Bacille Calmette Guerin (BCG) vaccination are enhanced in the immune suppressed [34, 35]. A mouse model also confirms these findings: higher proportions of undernourished mice exhibited bacterial dissemination to lymph nodes following vaccination with BCG [36].

Current guidelines for management of malnutrition do not include recommendations for adaptation of vaccines or schedules, and children with malnutrition are generally vaccinated according

to the same routine schedule as well-nourished children, except in the presence of severe acute illness [37]. The immune responses and reactogenicity (local and systemic reactions) to vaccines elicited in malnourished children compared to well-nourished children remains a poorly researched area, and more data are required to inform the question as to whether vaccination ought to be adapted in the context of malnutrition, given that it remains a leading cause of preventable childhood deaths globally. If vaccines do not perform equally well in the context of malnutrition, practices might require adaptation in children with malnutrition.

The period of growth faltering coincides with the delivery of routine vaccines within the Expanded Programme on Immunization (EPI), including yellow fever, measles and oral polio vaccines [37]. It already occurs around the period of introducing complementary feeds starting at 3 to 6 months of age in much of sub-Saharan Africa and is noticed in up to 50% of children attending infant welfare clinics [38-40]. This faltering persists well into the 5th year [38]. It is therefore particularly important to assess the vaccine responses in the context of nutrition in the second half of infancy (6-12 months), where important live vaccines are used. In many countries which fall into the African meningitis belt, the MenAfriVac vaccine a serogroup A conjugate meningococcal vaccine has also been introduced for this age group[41].

In clinical practice malnutrition is often measured using the World Health Organization (WHO) z-scores for weight for age, height for age and weight for height. These standards have evolved over the years and were developed using data collected in the WHO Multicentre Growth Reference Study. These guidelines were first published in 2006 based on data from 1990 collected from British children. The 2006 standards were based on breastfed infants from different ethnic groups. These standards define varying degrees of malnutrition as standard deviations from median values [42].

Factors such as age, [43-45] sex, [46, 47] geographical location, [45, 48, 49] seasonality [50] and pre-existing immunity [51] may all have additional impact on vaccine-induced protection. These factors therefore ought to be considered as covariates when assessing vaccine responses.

In the current era of precision medicine and precision vaccinology with a drive to tailor interventions to specific populations rather than have generic ‘one size fits all’ interventions, it is important to explore these questions to guide the use of available interventions or production of newer interventions.

1.3 **Yellow fever**

Yellow fever is among the most feared and disruptive epidemic-prone diseases. Endemic in sub-Saharan Africa and the Americas, yellow fever is caused by a flavivirus transmitted through the bite of infected *Aedes aegypti* mosquitoes [52]. The intermediate host is the rhesus monkey. [53] The incidence of this potentially severe haemorrhagic disease has markedly reduced since

the introduction of vaccines against the virus. There have however been several recent outbreaks in some cases beyond the tradition regions known for these outbreaks [52, 54]. The vast majority - ninety five percent of the remaining cases occur in Africa [55].

Effective vaccines against yellow fever virus have been available for routine use in yellow fever endemic regions of Africa and Asia since the year 1937 [53]. All currently available yellow fever vaccines are live attenuated vaccines, which are known to be highly immunogenic with a single dose generally providing long term protection [53]. The primary correlate of protection is neutralizing antibody, though cell mediated and innate immune responses have also been proposed to play a role in protection [56]. It may however be that protection is not universally consistent across different geographical settings and age groups. A study in Brazilian infants reported varying seroconversion rates in children of different ages [44]. Previous analysis of data from the MenAfriVac trials also noted varying seroconversion rates between countries [57]. Seroconversion rates of 93% immediately following vaccination have been reported from adults in Brazil, but lower rates of 87 % for yellow fever vaccine given alone and 70% when given with Measles, Mumps Rubella (MMR) vaccine were reported in Brazilian children in the immediate post vaccination period [45, 48]. In contrast to this, a previous study from the Gambia conducted in well-nourished children as part of a trial of the immunogenicity and safety of inactivated polio vaccine (IPV) reported seroconversion rates for yellow fever of 95% and above when given alone or in conjunction with varying combinations of Measles, MMR vaccines and IPV [49].

The current World Health Organization (WHO) guidance for the use of yellow fever vaccines recommends a single dose of yellow fever vaccine for life and fractional doses in outbreak situations where vaccine availability is limited. This is in contrast to previous recommendation of repeated doses every ten years [58-61]. A number of research priorities have been identified by WHO to ensure continued optimal use of this otherwise highly effective vaccine, including the conduct of further studies to identify at risk groups who may require a booster dose, to include infants and immunocompromised individuals such as those with HIV infection; and examination of vaccine responses where yellow fever vaccines have been administered concomitantly with other live vaccines, in particular the MMR vaccine [58].

Whether immunogenicity of yellow fever vaccine could also be affected by malnutrition- has not been systematically investigated to date. The yellow fever vaccine is given routinely at 9 months in most of the developing world - an age when growth faltering and malnutrition are common problem [28, 30]. New threats from yellow fever including outbreaks have recently emerged [54]. This includes, the risk of urban yellow fever infection due to transmission of the virus by the vector, increasing in Africa, and the potential for urban YF returning to South America. Both present serious potential public health problems especially in settings with large population density

[55]. Geographical areas affected by yellow fever have also recently expanded to include areas previously considered risk free [62].
The recent changes to vaccination recommendations for yellow fever at a time of changing threats, calls for further assessments of potential impact of these important and prevalent co-factors in order to continue optimal use of this otherwise highly protective vaccine.

1.4 Measles

Measles is a highly contagious systemic viral disease still responsible for significant mortality and morbidity and has the potential to lead to major epidemics [63, 64]. The illness is caused by a negative-sense RNA virus with a non-segmented genome and a lipid envelope of the genus morbillivirus and the family Paramyxoviridae. The most recent concerns around measles include outbreaks in several countries during 2019 and still ongoing in June 2019 having first being noted in late 2018 [65-67]. Over 60,000 individuals have been infected in 3 countries (Ukraine, Philippines and Brazil) alone with 340 deaths as at 19th March 2019 [65, 66]. The first four months of 2019 saw a 700% increase in measles cases reported from the WHO Afro region compared to the same period in 2018 [67]. Outbreaks have occurred in all WHO regions [66] and the containment of measles has gained high prominence in public health institutions across high and low income countries alike in recent months [66, 68].
Live attenuated vaccines are available against measles and these vaccines are safe and immunogenic and provide long lasting protection [63, 69]. Two doses are recommended to susceptible children and adults either as measles alone or concomitantly with other antigens such as mumps and rubella as combination or single antigen vaccines and with varicella vaccine [37]. Reaching all children with 2 doses of measles containing vaccine should be routine for all country's immunization programmes with elimination possible at coverage rates over 95% [37]. In the most recent outbreaks over half of the cases which occurred in most countries were in unvaccinated individuals or those with unknown vaccination status [65, 70].
In much of the developing world, measles vaccine is administered at 9 months of age with a booster dose given at the age of 15 month in some countries [37]. The immune response to measles vaccine is age dependent, partially inhibited by maternal antibody (Ab) and involves induction of both Ab and T cell responses [63]. Neutralizing antibodies are considered a correlate of protection [63, 71].

Measles vaccine antibody responses have previously been shown to be affected by anthropometric parameters [72]. This study described weight for height/length as impacting antibody concentrations 1-year post vaccination. Of the children with normal weight for height/length, 75% had serum IgG concentrations above the defined threshold for protection compared to 59% of those with reduced weight for height/length [72]. It is not clear what cut off z score was used for this study however, and other measures of under nutrition were not assessed.

WHO's 2017 position paper on measles vaccines calls for research targeted at programmatic issues for delivery of measles containing vaccines and for special groups to target with vaccination.

2 Rationale and Objectives

We hypothesize that due to possibly subtle underlying immune suppression in malnourished children, routine vaccines given to this group of children will be less immunogenic compared to their well-nourished counterparts. Such poor responses may lead to inferior disease protection particularly in terms of quality and longevity of vaccine response, leaving an already vulnerable group of children more vulnerable to preventable infectious disease.

We chose to investigate the relationship between yellow fever and measles vaccines antibody responses and anthropometric parameters in children nine to 11 months of age, an age well documented to be particularly associated with malnutrition and growth faltering in the developing world, [28, 30] and an age where important live vaccines are routinely given. We had access to a data from several large clinical studies conducted among children in sub-Saharan Africa. We examined differences in seroconversion rates and quantitative titres to yellow fever and measles by age, sex and country, and we assessed the effect of season of vaccination and pre-existing antibody titres in a set of data available to us from these studies, as outlined below.

2.1 Primary objective:

To explore differences in immune responses to vaccination in well-nourished infants and infants with under- nutrition

2.2 Secondary objectives:

1. To assess differences in immune responses based on degree of under nutrition.
2. To assess the longevity of vaccine responses.

3 Methods and Materials

To address the question of a potential link between anthropometric and co factors to immune responses to yellow fever and measles vaccination, we analysed available data from three of the large-scale vaccine trials that led to licensure of a new vaccine against meningococcal A meningitis (MenAfriVac/PsA-TT) and in which our research organisation was a collaborator. I was a co-investigator for 3 of these studies.

The Meningitis Vaccine Project (MVP), a partnership between the World Health Organization (WHO) and the Program for Appropriate Technology in Health (PATH) conducted these studies

in the African meningitis belt which extends from Ethiopia to Senegal through a public-private partnership with the Serum Institute of India.[41, 73].

3.1 Materials

In order to achieve licensure of the MenAfriVac (PsA-TT) vaccine, 9 studies were conducted in individuals 14 weeks to 35 years within the African meningitis belt. Participants were vaccinated with MenAfriVac and followed up for up to 5 years between 2005 and 2012. These studies thus resulted in collection of a wide range of clinical and laboratory data and samples. This data and serum is available under a memorandum of understanding between PATH and Serum Institute of India to be accessed by investigators wishing to conduct relevant analysis with the available data and samples.
To carry out this analysis I accessed data and serum samples as available from the studies that included children within the targeted age group of 9 to 11 months at the time of receiving a vaccination. This age group was targeted as it represents a period when routine vaccines are given after the onset of documented growth faltering. Data and serum from 3 studies across 3 countries has been utilized. The original studies are described in brief below:

3.1.1 Original study designs

Study PsA-TT 004 conducted in Navrongo, Ghana:

Between November 2008 and August 2009, 1,200 participants were enrolled at 14-18 weeks of age and depending on the group to which the participant was randomized, first dose of the study vaccine was received at 14 weeks, 9 months, or 12 months of age. Children received one or two doses of different dosages of MenAfriVac. The control group was vaccinated with MenAfriVac at the end of study but there was no post-vaccination blood draw.
For the purposes of my analysis only data from infants who received, measles, yellow fever and MenAfriVac vaccines at age 9 to 11 months at standard concentration was utilized. Data from the pre-vaccination and 28 days post vaccination time point were accessed for yellow fever vaccine responses.

Study PsA-TT 007 conducted in Bamako, Mali:

Between March 2012 and December 2012, participants were enrolled and randomized to receive one or two doses of different dosages of MenAfriVac. All children were enrolled at 9-12 months of age. For the purposes of my analysis only data from infants who received, measles, yellow fever and MenAfriVac vaccines at standard concentration was utilized. Data from the pre-vaccination and 28-day post vaccination time point were accessed for both yellow fever and

measles vaccine responses. For responses to measles vaccine, data was also available for IgG responses to measles 6 months post vaccination.

Study PsA-TT 002 conducted in Basse, The Gambia and Bamako, Mali:

Between September and November 2006, six hundred and one (601) twelve to twenty-three-month-old toddlers were recruited in The Gambia and Mali. The infant received in a 1:1:1 ratio a dose of either MenAfriVac, the licenced polysaccharide tetravalent vaccine containing serogroup A, C, W and Y strains (PsACWY) or Haemophilus influenza type b vaccine conjugated to tetanus toxoid (Hib-TT). A second vaccine dose was given 6 months later again randomized in a 1:1:1 fashion creating a total of 9 groups. For the purposes of my analysis, data from an extended time point 4 to 5 years post the original study was assessed for yellow fever vaccine responses (see below). All infants enrolled had completed the primary series of EPI vaccines according to local schedule prior to enrolment. Blood samples were collected prior to vaccination in the study, 4 weeks post vaccination, prior to secondary vaccination, and approximately 1, 2- and 5-years post vaccination. The 5-year time point was the Pers-002 study (see below).

Study Pers-002-003 conducted in Basse, The Gambia and Bamako, Mali:

Participants who participated in 2 studies PsA-TT-002 and 003 and were enrolled between October 2011 and April 2012 to have a single blood draw to measure immune persistence since their last study visit (up to 4-5 years after first vaccination).
For the purposes of my analysis, data from the Pers-002 study were accessed as these children had originally been enrolled at age 12 – 23 months shortly after routine yellow fever vaccination – N= 481. An enrollment criterion for the original PsA-TT 002 study, for which this study was a follow up, was the requirement to have received all required EPI vaccines as dictated by the local schedules. For both countries this included concomitant measles, yellow fever and OPV vaccines at the age of approximately 9 months of age.
The serum from these individuals will also be utilized to assay iron and vitamin D at the point just prior to vaccination in the initial studies at 12 - 23 months of age. These nutritional markers will then be correlated to vaccine responses for my post-doctoral research.
For the MenAfriVac persistence study, a single serum sample was collected from the original participants between November 2011 and January 2012, approximately 5 to 6 years post receipt of yellow fever vaccine within routine EPI programmes.
For my analysis, serum from these participants was utilized to assay IgG antibody to yellow fever as a follow up to findings from the yellow fever IgG concentrations noted in the Malian and

Ghanaian cohorts. Of note, there were no baseline titres or anthropometry at the time of vaccination for comparison.

3.2 Data/Sera Retrieval Process:

Prior to receiving the data and samples, I completed data/sera access forms and sought the written consent from the original investigators of these studies to access the data. These forms were then reviewed by a team at the Program for Appropriate Technology in Health and the Serum Institute of India. After obtaining these permissions, I applied for and received ethical approvals from the local ethics committees in all the countries where the data and samples had been collected to use the available data/samples for this purpose. This was followed by secure transmittal of the data to me and sample shipments from the sera bank Laboratorio di Epidemiologia Molecolare facility affiliated to the Dipartimento di Fisiopatologia, Medicina Sperimentale Sanità Pubblica of the University of Siena in Italy (UNISI) where all the serum samples were held.

3.3 Nutritional assessments:

Nutritional parameters for both groups were defined using the World Health Organization (WHO) z-scores for weight for age, height for age and weight for height just prior to vaccination.[42]. These standards were published in 2006, based on breastfed infants and appropriately fed children raised in optimal conditions and from different ethnic groups. These standards define varying degrees of malnutrition as standard deviations from median values. [42]

3.4 Nutrition and antibody response to yellow fever vaccine

We investigated the relationship between yellow fever and measles vaccine antibody responses and anthropometric parameters in children nine to 11 months of age. We examined differences in seroconversion rates and quantitative titres by age, sex and country, and we assessed the effect of season of vaccination and pre-existing antibody titres

3.4.1 Participants

Details of the trials are provided above. In summary, participants for the comparison of vaccine responses to anthropometry, were originally enrolled into two trials conducted across the African meningitis belt to test the safety, immunogenicity, dose response and schedule of MenAfriVac. This vaccine has recently been introduced across the African meningitis belt in a bid to eliminate periodic meningococcal A epidemics. The trials were conducted between 2008 and 2013. The trial in urban Mali recruited 1,500 infants aged 9-18 months between March 2012 and

September 2013, while the study in rural Ghana recruited 1,200 infants aged 14 -18 weeks between Nov 2008 and May 2012 [41, 73].

For this analysis, we included data from all 393 participants who had received yellow fever vaccine at 9 to 11 months of age from clinical trial teams and had results available for yellow fever antibody titres at 2 time points pre and post vaccination.[74, 75] Serum samples were collected pre- and 28 days post vaccination.

All infants had received a single dose of yellow fever vaccine (see below) from the trial teams. In addition, they all received a single dose of measles (Serum institute of India) and Oral Polio Vaccine (GlaxoSmithKline) together with MenAfriVac^{TM} (Serum Institute of India).

Both yellow fever vaccines were live attenuated freeze-dried vaccines containing ≥ 1000 lethal dose 50 units per dose. The FioCruz yellow fever 17D strain sub strain 17DD vaccine (used in Ghana) has an antigen concentration of between 4·34 $\log_{10}$ plaque-forming units (PFU) and 4·56 $\log_{10}$ PFU per dose. The Federal State Unitary Enterprise of Chimakov Institute of Poliomyelitis and Viral Encephalitis Russian Academy of Med Sciences 17D strain (used in Mali) has a vaccine concentration between 4·5 $\log_{10}$ plaque-forming units (PFU) and 4·7 $\log_{10}$ PFU per dose.

3.4.2 Laboratory methods:

All yellow fever vaccine responses had originally been measured using a neutralization assay optimised and available at the Robert Koch-Institute (RKI) in Berlin. The assay was done using the yellow fever -17D target virus strain in a concentration of 100 tissue culture infectious disease dose, 50% ($TCID_{50}$) per well produced at RKI. Dilutions yielding ≥ 50% neutralization after 5 days were expressed as neutralization titres based on the dilution which attained this [57]. To mitigate against batch effects from different runs of the samples, a human serum sample with a medium neutralization titre was run as an internal control for each batch of samples run. If this control did not react as a medium titre the batch of samples was rerun. The threshold of 1:5 was considered as protective antibody titres while seroconversion was defined as at least a four-fold increase in antibody titre [71, 76]. Titres were measured as a ratio. All yellow fever assays were carried out in the same laboratory, using identical procedures.

3.4.3 Statistical methods:

Where applicable, antibody titres were normalized by log 2 transformation. Uni and multivariable mixed effects models were applied with random effects for country, adjusting for age, sex, season of vaccination and pre-vaccination titres as fixed effects. Separate models were run for seroconversion (binary outcome), raw post-vaccination antibody titres (continuous) and protection post-vaccination (binary) using logistic regression for binary outcomes and linear regression for

continuous outcomes. Means, medians, proportions and odds ratios were calculated along with their corresponding 95% confidence intervals. An alpha error level of 5% was used to judge significance. Where applicable data were log transformed to normalize them. Data analysis was done using Stata version 14.2 [77].

3.5 Nutrition and response to measles vaccine

3.5.1 Participants:

For measles vaccine responses, data from 1,105 children was analysed at baseline and 28 days post vaccination. Data was also available from 1,081 of these children 6 months post vaccination and was examined at this time point. These infants were all from Mali.

Data from infants who had received measles and yellow fever vaccines concomitantly at 9 to 11 months of age was reanalysed to correlate nutritional status at the time of vaccination to subsequent response to these vaccines.

The infants had originally participated in a randomised controlled clinical trial conducted in Mali, to test the safety, immunogenicity and dosing schedule for the MenAfriVac vaccine. All infants had received measles and yellow fever vaccines from the trial teams during their participation in the trial at 9 – 11 months of age. All responses presented follow 1 dose of the vaccine in question. No booster doses had been given.

Infants the study received measles vaccine produced by Serum Institute of India Limited.

3.5.2 Lab methods:

Measles responses were measured by measles specific IgG concentration using a standard measles specific IgG ELISA kit IGG Virion Serion at UNISI. Batch effects (day to day variations and deviations) were minimized by running a test sample with each batch of samples run. In addition, multiplication of the current measured value obtained with a sample with a correction factor F was done. This procedure served to adjust the current test level of the user with a lot-specific standard curve. Values which fell below the lower limit of detection were assigned a value of 50mIU/mL.

$$F = \frac{\text{OD of reference value (of standard serum)}}{\text{OD of current value (of standard serum)}}$$

OD = optical density

A threshold of 120mIU/ml was considered as the cut off for protection [71, 78-80]. This is defined as a definite correlate of protection given that the presence of this antibody concentration

is almost always associated with protection from disease. [71] Seroconversion was defined as at least a 4-fold rise in antibody concentration from baseline [71, 79].

3.5.3 Statistical methods:

Statistical methods similar to those for responses to yellow fever were used. However, since all children belonged to the same cohort, there was no need to use mixed effects models as in the yellow fever analyses. In addition, attempts to perform multivariable regression analysis of z scores as predictor variables of the binary threshold of protection outcome adjusting for age and sex resulted in null cells, thus only pre-vaccination titres and season of vaccination was adjusted for, when this parameter was considered.

4. Results

4.1 Nutrition and antibody response to yellow fever vaccine

Pre- and post-vaccination data from a total of 393 children were utilized to explore the relationship between anthropometry and antibodies to yellow fever.
The mean age in this cohort was 9.22 (95%CI = 9.17 – 9.28) months. The mean antibody titres pre-and post-vaccination were 3.12 (2.96 - 3.27) and 35.48 (31.23 – 39.72) respectively. The mean fold change post vaccination was a 12.93 (11.18 – 14.68) fold rise in antibody titre. Other cohort characteristics are detailed in Table 4.1.

Table 4.1: Cohort characteristics – yellow fever antibody responses.

N = 393		All		Male (54%)		Female (46%)	
Country	**Variable**	**Mean (95% CI)**	**Median (IQR)**	**Mean (95% CI)**	**Median (IQR)**	**Mean (95% CI)**	**Median (IQR)**
Both							
N = 393							
	Age (months)	9·22 (9·17, 9·28)	9·00 (9·00, 9·00)	9·26 (9·18, 9·34)	9·00 (9·00, 9·00)	9·21 (9·13, 9·29)	9·00 (9·00, 9·00)
	Weight (kg)	8·19 (8·09, 8·28)	8·10 (7·50, 8·80)	8·35 (8·22, 8·47)	8·30 (7·80, 8·90)	8·00 (7·86, 8·14)	8·00 (7·30, 8·70)
	Height (cm)	70·9 (70·7, 71·1)	71·0 (69·1, 72·0)	71·2 (70·9, 71·6)	71·0 (70·0, 73·0)	70·5 (70·2, 70·9)	70·2 (69·0, 72·0)
	Weight for age z-score	-0·54 (-0·64, -0·44)	-0·72 (-1·42, 0·00)	-0·71 (-0·84, -0·57)	-0·75 (-1·32, -0·01)	-0·35 (-0·49, -0·20)	-0·33 (-0·99, 0·27)
	Height for age z-scores	-0·24 (-0·34, -0·13)	-0·23 (-0·88, 0·38)	-0·47 (-0·61, -0·34)	-0·43 (-1·15, 0·01)	0·05 (-0·11, 0·20)	-0·06 (-0·60, 0·77)
	Weight for height z-score	-0·71 (-0·83, -0·60)	-0·61 (-1·21, 0·08)	-0·78 (-0·95, -0·62)	-0·79 (-1·50, -0·03)	-0·63 (-0·79, -0·47)	-0·69 (-1·30, 0·12)

YF Ab (base-line) = a	3·12 (2·96, 3·27)	2·00 (2·00, 4·00)	3·11 (2·89, 3·33)	2·00 (2·00, 4·00)	3·12 (2·90, 3·34)	2·00 (2·00, 4·00)
YF Ab (28 days post vac) = b	35·48 (31·23, 39·72)	22·00 (11·00, 45·00)	38·74 (31·76, 45·71)	32·00 (11·00, 45·00)	31·61 (27·38, 35·85)	22·00 (11·00, 45·00)
YF Ab fold change a to b	13-fold (11, 15)	8-fold (4,16)	14-fold (11, 17)	11-fold (4, 16)	11-fold (10, 13)	8-fold (4, 16)
Mali						
N = 232	**All**		**Male – 56%**		**Female – 44%**	
Age (months)	9·34 (9·25, 9·42)	9·00 (9·00, 9·00)	9·36 (9·24, 9·47)	9·00 (9·00, 9·00)	9·32 (9·19, 9·45)	9·00 (9·00, 9·00)
Weight (kg)	8·31 (8·20, 8·42)	8·15 (7·80, 8·80)	8·45 (8·31, 8·58)	8·40 (7·90, 8·90)	8·13 (7·97, 8·30)	8·00 (7·50, 8·70)
Height (cm)	71·1 (70·7, 71·4)	71·0 (70·0, 73·0)	71·3 (70·9, 71·7)	71·0 (70·0, 73·0)	70·7 (70·3, 71·3)	71·0 (69·5, 72·0)
Weight for age z-score	-0·43 (0·86, -0·52)	-0·52 (-1·02, 0·09)	-0·61 (-0·76, -0·46)	-0·67 (-1·09, -0·11)	-0·21 (-0·38, -0·05)	-0·23 (-0·80, 0·41)
Height for age z-scores	-0·25 (1.02, -0·12)	-0·12 (-0·88, 0·35)	-0·51 (-0·67, -0·35)	-0·43 (-1·32, 0·01)	0·10 (-0·10, 0·30)	0·09 (-0·54, 0·77)
Weight for height z-score	-0·56 (1.01, -0.59)	-0·59 (-1·20, 0·00)	-0·63 (-0·81, -0·45)	-0·59 (-1·18, -0·14)	-0·49 (-0·68, -0·30)	-0·57 (-1·21, 0·12)
YF Ab (base-line) = a	3·35 (3·15, 3·56)	4·00 (2·00, 4·00)	3·32 (3·06, 3·57)	4·00 (2·00, 4·00)	3·40 (3·07, 3·73)	4·00 (2·00, 4·00)
YF Ab (28 days post vac) = b	41·32 (37.54, 44.72)	32·00 (22·00, 45·00)	43·27 (38·13, 48·42)	32·00 (22·00, 64·00)	38·42 (33·51, 43·33)	32·00 (22·00, 45·00)
YF Ab fold change a to b	15-fold (13, 17)	11-fold (8, 16)	15-fold (13, 17)	11-fold (8, 16)	13-fold (12, 15)	11-fold (6, 16)
Ghana N=161	**All**		**Male – 52%**		**Female – 48%**	
Age (months)	9·04 (9·01, 9·08)	9·00 (9·00, 9·00)	9·04 (9·00, 9·08)	9·00 (9·00, 9·00)	9·05 (9·00, 9·10)	9·00 (9·00, 9·00)
Weight (kg)	8·01 (7·84, 8·18)	7·90 (7·20, 8·80)	8·20 (7·95, 8·44)	8·00 (7·50, 8·90)	7·81 (7·57, 8·05)	7·70 (7·00, 8·40)
Height (cm)	70·6 (70·2, 71·0)	70·0 (69·0, 72·0)	71·1 (70·6, 71·6)	70·4 (69·5, 73·0)	70·1 (69·6, 70·7)	70·0 (68·5, 71·3)
Weight for age z-score	-0·70 (-0·88, -0·51)	-0·81 (-1·56, 0·07)	-0·84 (-1·11, -0·57)	-0·98 (-1·56, 0·00)	-0·53 (-0·78, -0·27)	-0·69 (-1·34, 0·17)
Height for age z-scores	-0·23 (-0·40, -0·06)	-0·43 (-0·88, 0·46)	-0·42 (-0·65, -0·18)	-0·70 (-1·15, 0·46)	-0·03 (-0·27, 0·21)	-0·06 (-0·68, 0·48)
Weight for height z-score	-0·92 (-1·13, -0·72)	-1·03 (-1·82, 0·03)	-1·00 (-1·32, -0·68)	-1·17 (-1·94, 0·09)	-0·82 (-1·10, -0·54)	-0·88 (-1·66, -0·01)
YF Ab (base-line) = a	2·77 (2·54, 3·01)	2·00 (2·00, 4·00)	2·81 (2·41, 3·22)	2·00 (2·00, 4·00)	2·75 (2·50, 3·01)	2·00 (2·00, 4·00)

YF Ab (28 days post vac) = b	27·30 (18·40, 36·22)	11·00 (6·00, 22·00)	32·35 (15·93, 48·76)	16·00 (6·00, 22·00)	22·60 (15·58, 29·61)	11·00 (6·00, 22·00)
YF Ab fold change a to b	11-fold (7, 15)	6-fold (2, 11)	13-fold (6, 20)	6-fold (2, 11)	9-fold (6, 12)	6-fold (3, 11)

95% CI = 95% confidence interval
IQR = interquartile range
YF Ab = yellow fever antibody
N = number of observations

4.1.1 Antibody responses and seroconversion rates for yellow fever

Mean and median antibody titres were statistically significantly higher in the Malian cohort both pre-and post-vaccination (p = <0·001 and p=0·002 respectively) as shown in Table 4.1 and Figure 4.1. There were no significant differences in age, weight, height and z-scores between countries for both sexes. The overall fold changes were not significantly different between the two countries or between sexes, although there was a trend towards higher fold changes among males (Table 4.1).

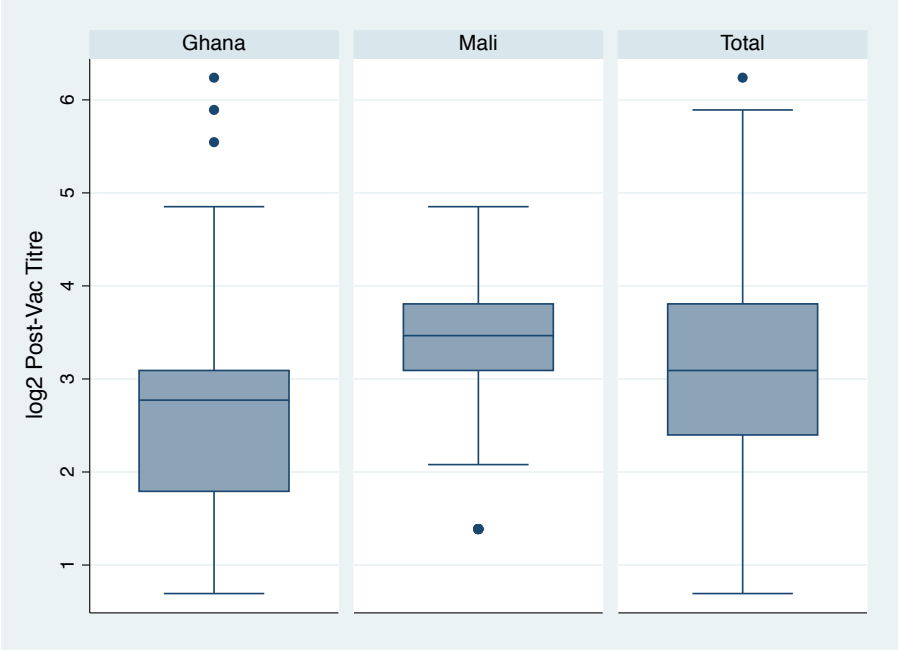


Figure 4.1: Post vaccination (28 days) anti-yellow fever Ab titre by country
Median and interquartile range
p value for country difference = 0.002.

Overall, 84% of children received their yellow fever vaccine at 9 months of age. The proportion of children who attained seroconversion when vaccinations were given at 9 months of age was significantly lower in the Ghanaian than in the Malian cohort (p <0·001), (Table 4.2). The odds of attaining a four-fold response in antibody titre were 8 times higher among the Malian cohort.

Table 4.2: Proportion of participants attaining a 4-fold increase (seroconversion) in anti-yellow fever antibody titre, stratified by age.

Age (months)	Overall		Ghana		Mali	
	N	Proportion (95% CI)	N	Proportion (95% CI)	N	Proportion (95% CI)
9	330	78·5 (73·7, 82·8)	152	63·8 (55·6, 71·4)	178	91·0 (85·8, 94·8)
10	36	80·6 (64·0, 91·8)	7	57·1 (18·4, 90·1)	29	86·2 (68·3, 96·1)
11	25	96·0 (79·6, 99·9)	0	--	25	96·0 (79·6, 99·9)
All	391	79·8 (75·5, 83·7)	159	63·5 (55·8, 71·0)	232	91·0 (86·5, 94·3)

N = number of observations
95% CI = 95% confidence interval
NB: Results recorded, as '0' for 2 participants so fold change could not be calculated
P value for difference in seroconverters between countries <0.0001
Odds of attaining a four-fold response in antibody titre was 7·97 times higher among the Malian cohort

4.1.2 **Influence of co-variates on antibody response rates to yellow fever vaccine**:

Of 28 children (9·5% of the cohort) who already had antibody titres above the defined threshold for protection prior to vaccination, between 96 and 99% were either well-nourished or only mildly malnourished respectively, according to their different WHO z-scores, compared to 88% of those who did have titres above the defined threshold.

There was no statistically significant association of failure to seroconvert with nutritional status, nor were there pronounced sex differences in seroconversion rates. There was a trend for higher antibody titres in boys, though this did not reach statistical significance (p=0·08; Table 4.1).

None of the WHO defined z-score parameters for anthropometry were significantly associated with seroconversion. (Table 4.3) The odds of attaining seroconversion were however slightly higher in children with higher z-scores with all odds ratios slightly above one particularly for height for age z score which is a measure of chronic malnutrition.

Table 4.3: Association between anthropometry z-scores and seroconversion (4-fold increase in anti-yellow fever antibody titres)

		Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Weight for height z-score					
(per unit)	391	1·02 (0·82 to 1·26)	0·8749	1·06 (0·85 to 1·33)	0·5855
Weight for age z-score					
(per unit)	393	1·13 (0·88 to 1·44)	0·3352	1·20 (0·92 to 1·56)	0·1716
Height for age z-score					
(per unit)	393	1·26 (0·98 to 1·63)	0·0697	1·31 (1·00 to 1·72)	0·0501

N = number of observations; OR = Odds ratio; 95% CI = 95% confidence interval

Results of separate uni- and multi-variable mixed-effects logistic regression, with random effects for country

Multi-variable model additionally adjusted for pre vaccination titres, age, sex and season.

Children with lower pre-vaccination titres had higher odds of attaining four-fold titre responses (seroconversion) post vaccination (Table 4.3).

Table 4.4 shows the full model, including all co-variates exploring the relationship of the co-variates with weight for height z scores. The other WHO z score criteria gave similar results.

Table 4.4: Association between weight for height z-score and seroconversion following yellow fever vaccine (N = 391; N-seroconverted = 312, % seroconverted = 79.8%)

				Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**N-pos.**	**%-pos.**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Weight for height z-score							
(per unit)	-	-	-	1.02 (0.82 to 1.26)	0.8749	1.06 (0.85 to 1.33)	0.5855
Log2 pre-vacc. titre							
(per unit)	-	-	-	0.31 (0.19 to 0.50)	<0.0001	0.30 (0.18 to 0.50)	<0.0001
Age							
(per month)	-	-	-	1.07 (0.56 to 2.04)	0.8317	0.99 (0.51 to 1.94)	0.9796
Sex							
male*	211	172	81.52	1.00 - -	-	1.00 - -	-
female	180	142	78.89	0.90 (0.53 to 1.53)	0.6992	0.92 (0.53 to 1.59)	0.7595
Season							
dry*	243	198	81.48	1.00 - -	-	1.00 - -	-
rainy	148	116	78.38	0.76 (0.45 to 1.31)	0.3285	1.04 (0.59 to 1.84)	0.8935

N = number of observations; N-pos. = number seroconverted; %-pos. = percent seroconverted;

OR = odds ratio; 95% CI = 95% confidence interval

** Reference stratum*

NB: Uni- and multi-variable mixed-effects logistic regression results, with random effects for country, multi-variable model adjusted for all variables shown.

None of the WHO z-score parameters for nutritional status was significantly associated with antibody titres above the defined threshold for protection following vaccination. Like in the analysis for seroconversion, the trend for higher responses was again observed at higher z-scores with the strongest association for the height for age z-score (Table 4.5 and 4.6).

Table 4.5: Association between anthropometry z-scores and defined threshold (1:5) for protection from yellow fever

		Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Weight for height z-score					
(per unit)	391	1·00 (0·77 to 1·30)	0·9843	1·02 (0·78 to 1·33)	0·8986
Weight for age z-score					
(per unit)	393	1·09 (0·81 to 1·47)	0·5488	1·13 (0·84 to 1·53)	0·4186
Height for age z-score					
(per unit)	393	1·24 (0·91 to 1·70)	0·1771	1·29 (0·93 to 1·80)	0·1265

N = number of observations; OR = Odds ratio; 95% CI = 95% confidence interval

Uni- and multi-variable mixed-effects logistic regression results, with random effects for country,

Multi-variable model additionally adjusted for pre vaccination titres, age, sex and season.

Table 4.6 : Association of weight for height z-score with threshold for protection from yellow fever (N = 391; N-pos. = 348; %-pos. = 89.00)

				Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**N-pos.**	**%-pos.**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Weight for height z-score							
(per unit)	-	-	-	1.00 (0.77 to 1.30)	0.9843	1.02 (0.78 to 1.33)	0.8986
Log2 pre-vacc. titre							
(per unit)	-	-	-	0.74 (0.42 to 1.30)	0.2972	0.71 (0.40 to 1.25)	0.2366
Age							
(per month)	-	-	-	1.38 (0.47 to 4.07)	0.5625	1.39 (0.46 to 4.17)	0.5586
Sex							
male*	211	191	90.52	1.00 - -	-	1.00 - -	-
female	180	157	87.22	0.76 (0.39 to 1.48)	0.4161	0.76 (0.39 to 1.48)	0.4164
Season							
dry*	243	214	88.07	1.00 - -	-	1.00 - -	-
rainy	148	134	90.54	1.25 (0.62 to 2.54)	0.5274	1.38 (0.67 to 2.84)	0.3796

N = number of observations; N-pos. = number with protection; %-pos. = percent with protection;

OR = odds ratio; 95% CI = 95% confidence interval

** Reference stratum*

NB: Uni- and multi-variable mixed-effects logistic regression results, with random effects for country, multi-variable model adjusted for all variables shown.

Table 4.6 shows the full model, including all co-variates exploring the relationship of the covariates with weight for height z scores. The other WHO z score criteria gave similar results. Pre-vaccination titres, age, sex and season were not significantly associated with protective antibody titres (Table 4.6).

Similarly, no significant associations were noted when fold change was considered as a continuous variable except that – like in the seroconversion analyses – pre-vaccination titres again significantly influenced the fold change in antibody titres (p <0·001, Table 4.7 and 4.8). The season of vaccination was correlated for univariate analysis only with slightly better fold change in the dry season.

Table 4.7: Association between anthropometry z-scores and post vaccination log2 fold change in anti-yellow fever antibody titres

		Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**Coef. (95% CI)**	**p-value**	**Coef. (95% CI)**	**p-value**
Weight for height z-score					
(per unit)	391	0·07 (-0·02 to 0·16)	0·1551	0·04 (-0·04 to 0·12)	0·3330
Weight for age z-score					
(per unit)	393	0·08 (-0·03 to 0·18)	0·1394	0·06 (-0·03 to 0·16)	0·1912
Height for age z-score					
(per unit)	393	0·04 (-0·07 to 0·14)	0·4985	0·05 (-0·04 to 0·14)	0·2727

N = number of observations; 95% CI = 95% confidence interval; Coef = coeficient

Uni- and multi-variable mixed effects linear regression results, with random effects for country,

Multi-variable model additionally adjusted for pre vaccination titres, age, sex and season.

Table 4.8: Association of weight for height z-score with log2 fold change in anti-yellow fever IgG antibody (N = 391; Mean = 2.959) using a linear regression model

			Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**Mean**	**Coef. (95% CI)**	**p-value**	**Coef. (95% CI)**	**p-value**
Weight for height z-score						
(per unit)	-	-	0·07 (-0·02 to 0·16)	0·1551	0·04 (-0·04 to 0·12)	0·3330
Log2 pre-vacc. titre						
(per unit)	-	-	-0.71 (-0.95 to -0.46)	<0.0001	-0.91 (-1.15 to -0.67)	<0.001
Age						
(per month)	-	-	0.23 (0.04 to 0.42)	0.0185	0.03 (-0.15 to 0.20)	0.7752
Sex						
male*	211	14.18	0.00 -	-	0.00 -	-
female	180	11.46	-0.13 (-0.34 to 0.08)	0.2309	-0.07 (-0.26 to 0.11)	0.4364
Season						
dry*	243	13.98	0.00 -	-	0.00 -	-
rainy	148	11.21	-0.24 (-0.45 to -0.02)	0.0326	-0.00 (-0.20 to 0.20)	0.9726

N = number of observations; Mean = mean log2 fold change; Coef. = coefficient; 95% CI = 95% confidence interval

** Reference stratum*

NB: Uni- and multi-variable mixed-effects linear regression results, with random effects for country, multi-variable model adjusted for all variables shown.

4.2 Nutrition and antibody responses to measles vaccine

Data from 1,105 infants from Mali was utilized to explore the relationship between measles vaccine responses and anthropometry z scores.

The mean age in this cohort was 9.3 months, with mean pre vaccination antibody concentrations of 57.87 (48.67 – 67.08) and mean fold change in antibody concentration at 28 days of 17.3 (16.8 – 17.8) with a mean concentration of 872.9 (848.7 – 897.2). The mean antibody concentration 6 months post vaccination was 665.0 (633.9 – 696.1). Other cohort characteristics are summarized in Table 4.9.

Table 4.9: Cohort Characteristics – measles vaccine response

	Overall		Male (51%)		Female (49 %)	
N = 1,105	Mean (95% CI)	Median (IQR)	Mean (95% CI)	Median (IQR)	Mean (95% CI)	Median (IQR)
Age (months)	9.34 (9.30 – 9.38)	9.00 (9.00 – 10.00)	9.36 (9.30 – 9.41)	9.00 (9.00 – 10.00)	9.32 (9.27 – 9.38)	9.00 (9.00 – 9.00)
Weight (kg)	8.34 (8.29– 8.40)	8.20 (7.70 – 8.90)	8.59 (8.52 – 8.67)	8.50 (8.00 – 9.10)	8.08 (8.00 – 8.15)	8.00 (7.40 – 8.60)
Height (cm)	71.2 (71.1 – 71.3)	71.0 (70.0– 73.0)	71.5 (71.5 – 71.9)	72.0 (70.0 – 73.0)	70.6 (70.4 – 70.8)	71.0 (69.0 – 72.0)
Weight for age z score	-0.37 (-0.43 to -0.32)	-0.42 (-0.98 to 0.18)	-0.46 (-0.54 to -0.38)	-0.53 (-1.09 to 0.10)	-0.28 (-0.36 to -0.21)	-0.31 (-0.87 to -0.27)
Height for age z scores	-0.15 (-0.21 to -0.09)	-0.06 (-0.88 to 0.46)	-0.31 (-0.40 to -0.23)	-0.43 (-1.00 to 0.46)	-0.03 (-0.05 to 0.11)	-0.06 (-0.60 to 0.77)
Weight for height z score	-0.55 (-0.61 to -0.49)	-0.57 (-1.18 to 0.07)	-0.56 (-0.64 to -0.47)	-0.57 (-1.18 to 0.03)	-0.54 (-0.62 to -0.45)	-0.56 (-1.20 to 0.12)
Measles Ab (baseline) = a	57.87 (48.67 – 67.08)	50.00 (50.0 – 50.0)	52.43 (47.79 – 57.06)	50.00 (50.0 – 50.0)	63.54 (45.37 – 81.72)	50.00 (50.00 – 50.00)
Measles Ab (28 days post vac) = b	872.9 (848.7 – 897.2)	820.4 (581.4 – 1089.6)	841.6 (809.1 – 767.9)	803.6 (567.3 – 1065.0)	905.6 (869.7 – 941.5)	847.9 (599.1 – 1134.8)
Measles Ab (6 months post vaccination N = 1,081) = c	665.0 (633.9 – 696.1)	535.7 (338.0 - 831.2)	623.7 (580.0 – 667.4)	494.3 (314.2 - 764.2)	707.3 (663.1 – 751.5)	578.5 (373.9 - 896.7)
Measles Ab fold change a to b	17.3 (16.8 – 17.8)	16.3 (11.6 – 21.7)	16.7 (16.1 – 17.4)	16.1 (11.3 – 21.3)	17.8 (17.1 – 18.5)	16.7 (11.8 – 22.4)
Measles Ab fold change a to c	13.5 (12.7 – 14.2)	10.6 (6.8 – 16.3)	12.4 (11.5 – 13.3)	10.0 (6.3 – 15.5)	14.5 (13.3 – 15.7)	11.4 (7.4 – 17.3)

Measles antibody expressed in mIU/ml
95% CI = 95% confidence interval
IQR = interquartile range
N = number of observations
NB: Pre-vaccination IgG concentration was 50.0 for 1,092 of the 1,105 observations thus the median values of 50.0 with 95%Ci of 50.0 to 50.0. The value of 50.0 was assigned if the value fell below the limit of detection

4.2.1 Antibody responses and seroconversion rates for measles vaccine

There was a general trend towards higher antibody concentrations in females compared to their male counterparts.

Of this cohort 98.3% attained seroconversion 28 days post vaccination. Similar proportions attained seroconversion across the different age groups (Table 4.10). The mean and median antibody concentrations were also similar across the ages. The majority (74.3%) of the cohort received the measles vaccine at 9 months of age.

Table 4.10: Proportion of participants attaining a 4-fold increase in antibody concentration (seroconversion) 28 days following measles vaccination.

Age (months)	N (%)	Proportion (95% CI)
9	822 (74.4)	98.3 (84.9 – 89.6)
10	190 (17.2)	98.4 (95.5 – 99.7)
11	93 (8.4)	97.9 (92.4 – 99.7)
All	1,105 (100)	98.3 (97.3 – 99.0)

N = number of observations
95% CI = 95% confidence interval

Twenty-eight days post vaccination only 2 children (0.2%) still had Ab concentrations below the definate threshold for protection. Both of these children were male and aged 9 months at vaccination. By 6 months post vaccination, however 30 children (2.8%) overall had concentrations below 120mIU/ml. Eighteen (60%) of these were male. There was no observed trend linked to the nutritional z scores for these children whose IgG antibody concentrations fell below the threshold at either time point.

4.2.2 Effect of covariates on antibody responses to measles vaccine

Pre-vaccination at 9 – 11 months of age, 99.6% of these children had anti-measles IgG less than the defined threshold for protection of 120mIU/ml. Of the 4 infants with concentrations above this defined threshold of protection, 1 was aged 10 months while the other 3 were 11 months of age. One of these children was male.

Table 4.11: Association between anthropometry z-scores and threshold for seroconversion (4-fold increase in anti-measles antibody concentrations)

		Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Weight for height z-score					
(per unit)	1,100	1.32 (0.83 – 2.09)	0.2366	1.50 (0.91 – 2.48)	0.1146
Weight for age z-score					
(per unit)	1,105	1.17 (0.72 – 1.91)	0.5301	1.15 (0.67 – 1.97)	0.6068
Height for age z-score					
(per unit)	1,105	0.88 (0.57 – 1.37)	0.5825	0.70 (0.43 – 1.15)	0.1599

N = number of observations; OR = Odds ratio; 95% CI = 95% confidence interval

Results of separate uni- and multi-variable logistic regression

Multi-variable model adjusted for pre vaccination concentrations, age, sex and season of vaccination.

Nineteen of the 1,105 children failed to attain seroconversion. Of this number 12 (63%) were male. There was no statistically significant association between the failure to seroconvert and nutritional status measured by anthropometry (Table 4.11)

The children with lower pre-vaccination concentrations had higher odds of attaining a four-fold rise in antibody concentrations (multivariable p =0.0114 Table 4.12) Similar results were obtained for the weight for age and height for age z scores.

Table 4.12: Association between weight for height z-score and seroconversion following measles vaccine (N = 1,105; N-seroconverted = 1,086, % seroconverted = 98.3%)

				Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**N-pos.**	**%-pos.**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Weight for height z-score							
(per unit)	-	-	-	1.32 (0.83 to 2.09)	0.2366	1.50 (0.91 to 2.48)	0.1146
Log2 pre-vacc. titre							
(per unit)	-	-	-	0.97 (0.93 to 1.01)	<0.0001	0.00 (0.00 to 0.27)	0.0114
Age							
(per month)	-	-	-	1.07 (0.56 to 2.04)	0.8317	1.22 (0.36 to 7.14)	0.5403
Sex							
male*	562	550	97.88	1.00 - -	-	1.00 - -	-
female	543	536	98.71	0.93 (0.46 to 1.88)	0.8451	2.56 (0.84 to 7.81)	0.0990
Season							
dry*	757	746	98.55	1.00 - -	-	1.00 - -	-
rainy	348	340	97.70	0.62 (0.25 to 1.57)	0.3165	0.47 (0.17 to 1.28)	0.1397

N = number of observations; N-pos. = number seroconverted; %-pos. = percent seroconverted;

OR = odds ratio; 95% CI = 95% confidence interval

** Reference stratum*

NB: Uni- and multi-variable mixed-effects logistic regression results, with random effects for country, multi-variable model adjusted for all variables shown.

The odds of attaining antibody concentrations above the defined threshold for protection were similar for all 3 z score parameters with no significant differences in attaining this threshold based on z scores (Table 4.13).

Table 4.13: Association between anthropometry z-scores and threshold for protection 28 days post vaccination (anti-measles antibody concentrations of 120mIU/ml)

		Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Weight for height z-score					
(per unit)	1,105	0.74 (0.20 – 2.75)	0.6586	0.70 (0.17 – 2.94)	0.6221
Weight for age z-score					
(per unit)	1,100	0.71 (0.18 – 2.84)	0.6233	0.73 (0.19 – 2.83)	0.6465
Height for age z-score					
(per unit)	1,105	0.85 (0.22 – 3.28)	0.8125	0.86 (0.22 – 3.38)	0.8347

N = number of observations; OR = Odds ratio; 95% CI = 95% confidence interval

Results of separate uni- and multi-variable logistic regression

Multi-variable model adjusted for pre vaccination concentrations, season.

There was no relationship between any of the examined covariates and the attainment of IgG antibody above the threshold defined for protection (Table 4.14). Similar results were obtained for the weight for age and height for age z scores.

Table 4.14: Association between weight for height z-score and attaining threshold for protection against measles (N = 1,105; N- above threshold = 1,103, % above threshold = 99.8%)

				Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**N-pos.**	**%-pos.**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Weight for height z-score							
(per unit)	-	-	-	0.74 (0.20 to 2.75)	0.6586	0.70 (0.17 to 2.94)	0.6221
Log2 pre-vacc. titre							
(per unit)	-	-	-	1.21 (0.00 to 918.05)	0.9549	1.37 (0.00 to 0.4608.66)	0.9394
Season							
dry*	757	746	99.87	1.00 - -	-	1.00 - -	-
rainy	348	340	97.98	0.46 (0.03 to 7.34)	0.5810	0.45 (0.03 to 7.23)	0.571

N = number of observations; N-pos. = number seroconverted; %-pos. = percent seroconverted;

OR = odds ratio; 95% CI = 95% confidence interval

** Reference stratum*

NB: Uni- and multi-variable mixed-effects logistic regression results, with random effects for country, multi-variable model adjusted for all variables shown.

None of the anthropometric z score parameters influenced the anti-measles IgG antibody concentrations measured as a continuous variable (Table 4.15).

Table 4.15: Association between anthropometry z-scores and log2 antibody concentrations post vaccination following measles vaccine

		Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**Coef (95% CI)**	**p-value**	**Coef (95% CI)**	**p-value**
Weight for height z-score					
(per unit)	1,100	-0.00 (-0.03 – 0.03)	0.8476	-0.00 (-0.03 – 0.03)	0.8233
Weight for age z-score					
(per unit)	1,105	-0.01 (-0.04 – 0.02)	0.4447	-0.01 (-0.04 – 0.02)	0.4341
Height for age z-score					
(per unit)	1,105	-0.01 (-0.04 – 0.01)	0.3259	-0.01 (-0.04 – 0.01)	0.3262

** Reference stratum*

N = number of observations; Coef = coeficient; 95% CI = 95% confidence interval

Results of separate uni- and multi-variable linear regression

Multi-variable model adjusted for pre vaccination IgG concentration, age, sex and season

Attainment of a higher anti-measles antibody concentration was significantly impacted by the pre vaccination concentration ($p < 0.001$) and sex ($p = 0.016$) both univariably and when adjusting for the other covariates and in multivariable analysis (Table 4.16). Females had significantly higher post-vaccination antibody concentrations and lower pre-vaccination concentration resulted in lower post vaccination concentrations.

Table 4.16: Association between weight for height z score and log2 anti-measles IgG concentrations (N = 1,105; Mean = 872.9)

** Reference stratum*

			Univariable (crude)		Multivariable (adjusted)	
Co variate	**N**	**mean**	**Coef (95% CI)**	**p-value**	**Coef (95% CI)**	**p-value**
Weight for height z score	-	-	-0.00 (-0.03 – 0.03)	0.8476	-0.00 (-0.03 – 0.03)	0.8233
Log2 pre-vac IgG concentration (per unit)	-	-	0.09 (-0.16 – 0.34)	<0.001	0.25 (0.13 – 0.37)	<0.001
Age						
(per month)	-	-	-0.12 (-0.21 to -0.02)	0.2382	0.04 (-0.04 – 0.12)	0.3357
Sex						
male*	562	841.0	0.00 - -	-	0.00 - -	
female	543	904.1	0.02 (-0.10 – 0.14)	0.0162	0.07 (0.01 – 0.13)	0.0175
Season						
dry*	757	852.9	0.00 - -	-	0.00 - -	
rainy	348	913.6	-0.00 (-0.13 – 0.13)	0.1273	0.05 (-0.01 – 0.12)	0.1058

N = number of observations; Coef = coeficient; 95% CI = 95% confidence interval

Results of separate uni- and multi-variable linear regression

Multi-variable model adjusted for pre vaccination IgG concentration, age, sex and season

Similar results as obtained 28 days post vaccination, were obtained at the extended time point 6 months post vaccination for all the methods of assessing response and for all anthropometric measures. The effects of pre-vaccination concentrations on the responses at 6 months were however less marked (p = 0.01 and 0.07 respectively).

5. Discussion and conclusions

We examined the impact of nutritional status and other selected covariates on antibody responses to yellow fever and measles vaccine measured within 28 days of receiving the yellow fever and measles vaccine in infants from rural and urban Africa. Three hundred and ninety-three infants were studied for yellow fever responses and 1,105 for measles responses. For the measles response we also report on 1,081 infants at 6 months post vaccination.

5.1 Yellow Fever

Prior to vaccination as expected, mean antibody titres for this cohort were below the defined threshold of protection of 1:5. [71] This finding suggests that the vast majority of children between the ages of 9 and 11 months are likely susceptible to yellow fever in the endemic setting of Ghana and Mali since neutralizing antibodies titres are considered the correlate of protection for yellow fever. [58-61] On the other hand, almost 10% of the infants did have pre-vaccination antibody titres above 1:5, indicating either natural exposure or the effect of persisting maternal antibodies, due to prior exposure or vaccination of the mother within routine vaccination programmes or prior natural exposure of the child to the virus. Almost all children (96%) in the small group with pre-vaccination titres above protective threshold were adequately nourished compared to 87% of those below the threshold. This may be a chance finding or suggest that mounting an adequate response to natural exposure may be linked to adequate nutrition. To our knowledge a possible relationship between nutrition and natural immunity has not been previously reported in the literature but larger cohorts would be required to confirm this finding.

The overall antibody titres and fold changes in response to yellow fever vaccination in our cohort were not significantly affected by the nutritional status as characterised by weight for age, height for age or weight for height z-scores. This finding is very reassuring, as it implies that even malnourished children can mount a protective response to this vaccine. It is a limitation of this study, however that the number of children with z scores in the severe malnutrition range was very low in our cohort, and the question of nutrition and response to this vaccine may still benefit from more detailed testing in this specific group. This is important, given that they make up a larger proportion [28] (up to 50% malnourished to some degree) [25, 30] of children receiving vaccines in routine EPI programmes compared to clinical trials, where malnutrition is often an exclusion criterium. Most of the malnourished children in the MenAfrivac trials happened to develop growth faltering during the trial, in line with the usual observation of malnutrition occurring at the time of weaning.

However, the trend for improved seroconversion especially in children with higher z-score for height for age (multivariable $p = 0.0501$) indicates that chronic malnutrition which this parameter measures, may play a role, and hence it is important to assess yellow fever responses in a larger group of children with severe/chronic malnutrition. In addition to measuring vaccine titres and rates of seroconversion, functional antibody studies and more detailed investigations of B cell memory and repertoire might also be needed to conclude on the impact of nutritional factors on long-term immunity and protection. In addition, nutritional factors other than anthropometry such as micronutrient deficiencies might also play a role in vaccine responses. The potential clinical significance of all these factors needs to be assessed in longitudinal cohort studies or as part of systematic routine surveillance systems.

Examining the longevity of yellow fever antibody titres would be of interest given that a recent systematic review suggests that the longevity of vaccine response may be impaired when vaccines are given to the malnourished [28]. This question is particularly important considering WHO's recent recommendation of changing from 10 yearly doses of YF vaccine to a single dose of yellow fever vaccine to achieve life-long protection. In addition, there are a number of ongoing studies to assess if full protection can be achieved by fractional dosing, driven by the shortage of the yellow fever vaccine supplies.

Following vaccination, over 79% of the overall cohort attained seroconversion rates of a four-fold rise in antibody titres in our cohorts. This is lower than previous reports of 95% seroconversion in The Gambia when yellow fever vaccine was administered concomitantly with measles, rubella and IPV vaccines [49]. When seroconversion rates were disaggregated by country, the seroconversion rates in Mali alone were more comparable to this prior finding. A remarkable finding which has also been previously reported [57] is the marked difference in seroconversion rates between the Ghanaian and Malian cohorts: barely 60% of the Ghanaian infants attained seroconversion compared to roughly 90% of the Malians. This is especially striking as pre-vaccination titres were only slightly lower in Ghana. Lower pre vaccination titres has already been shown to be related to higher seroconversion suggesting that this difference between countries may not be purely due to an environmental effect due to the differences in location from which these participants were recruited. If differences in antibody longevity were to be shown between those with lower seroconversion rates and the group with adequate seroconversion, a booster dose needs to be considered to protect these individuals.

Given that up to 20% of infants overall do not achieve seroconversion, as shown in our dataset, the protective efficacy of the vaccine at a population level within the sub-region might also be jeopardised, especially if population coverage rates are suboptimal as they often are. The situation would be even more concerning in the Ghanaian cohort where close to 40% did not seroconvert.

It is of note that the children in these 2 countries received different preparations of yellow fever vaccines, which might have been a significant contributing factor. The two yellow fever vaccines contain antigens with slightly different genomic sequences which are not considered of identical genotype or phenotype [81]. They also differ in the passage level [82] and envelope protein glycosylation sites. Although these vaccines have been compared for non-inferiority in clinical evaluation, they have never been compared directly side by side [83]. Our findings support a need for such side-by-side evaluation, given that countries usually decide what vaccine to use for logistical and cost reasons. Such data could influence decision-making with regards to what vaccine to use.

Another potential contributing reason for the observed inter country variability could be the fact that the children in Ghana were from a rural area while those in Mali were recruited from an urban setting. This may suggest that other nutritional (other than anthropometric measures) or environmental variables including differential prior exposure to other infections or infestations which may include exposure to other flavi viruses play a role. Prior flavi virus exposure has been linked to limited severity of yellow fever infection[84] and may also impact response to the yellow fever vaccine.

Examining other vaccine responses between these 2 cohorts particularly where the same vaccine was used, and likely causes for these differences or their generalisability should be further explored. These comparisons would be useful to better delineate these marked differences in proportion of seroconversion.

The season of vaccination had no impact on responses in our cohort, which differs from some previous studies showing poorer vaccine response during the lean (rainy season) months especially in rural settings [50].

It is important to note that unlike the responses observed in relation to some other vaccines [46, 47], there were no significant sex differences in the fold change or quantitative response to yellow fever, as also reported previously [57]. There was however a trend towards higher responses in boys. This is different from previous reports of trends for higher responses in females to vaccines in general due to immunological difference between the sexes,[46, 47] but in accordance with previous findings from Pfister et al. who found higher antibody response in males for two yellow fever vaccines [83]. Taken together, these findings suggest that sex differences in vaccine responses are vaccine specific and probably quite complex.

The odds of attaining a fourfold response in antibody titre were significantly higher in infants who had lower pre-vaccination antibody concentrations. This is not surprising as the magnitude of response needed to attain a fourfold response would be much lower in children with lower pre-vaccination titres. This finding is also in keeping with previous results from 12 to 23-month-old Gambian children following receipt of the MenAfriVac vaccine [51]. In addition, the odds of attaining an antibody titre post vaccination above the defined threshold for protection was significantly higher in the Malians, which is probably partly a consequence of the higher pre-vaccination antibody titres in these children and related to the factors discussed above.

Limitations:

Many more infants in this study (84%) received the yellow fever vaccine at 9 months of age and only Mali vaccinated infants aged 11 months. There were relatively few malnourished children. Thus, comparisons between the ages and nutritional groups would benefit from reassessment in studies specifically powered for these outcomes.

5.2 Measles

We examined the impact of nutritional status and other selected covariates on antibody responses to measles vaccine measured within 28 days of receiving the measles vaccine in 1,105 infants from urban Africa. Data was also available at an extended time point 6 months post vaccination for 1,081 infants.

Prior to vaccination the majority of infants had serum antibody titres well below the defined threshold for protection of 120mIU/ml [78-80, 85]. This finding confirms that virtually all children in this region are susceptible to measles infection at 9 to 11 months of age, supporting the need for timely vaccination. The almost total absence of concentrations above the defined threshold for protection may support recent studies advocating for earlier measles vaccination given the low sero prevalence of measles antibody concentrations [80, 86] and the appreciable immune responses to vaccination in children younger than 9 months [87, 88]. This suggestion of a need for earlier vaccination may also be supported by a study which documented that girls loose maternal antibody faster than boys and may be more susceptible to infection prior to 9 months of age [88]. Our findings also do not suggest better responses with increasing age with similar seroconversion rates at 9, 10 and 11 months. While this may be different prior to 9 months of age, early measles vaccination may be an important consideration given the recent worldwide measles outbreaks including in children too young to have been vaccinated in routine programmes [66, 70]. The use of boosting doses currently recommended by WHO [37] should also ensure that adequate antibody concentrations are ultimately sustained to provide long lasting protection especially where initial vaccination happens early.

None of the anthropometric measures for malnutrition significantly influenced the antibody response to measles vaccine. An impact of nutrition measured by anthropometry on measles vaccine responses has previously been documented [72] in children from Uganda based on weight for height/length z scores. The Ugandan study reports infant wasting as being associated with lower measles specific IgG antibody concentrations. This finding differs from our own data, and it may suggest that there are regional or genetic differences in the factors influencing vaccine responses. Its clinical significance will need to be further explored but it is reassuring that protective concentrations are generally being attained.

The significantly higher antibody concentrations post vaccination in girls which we observed has been previously reported in a cohort of similar age from Spain [89]. Similar patterns were also observed for rubella and mumps vaccines [89], and a study of the now discontinued Edmonston-Zagreb strain of measles vaccine reported excess mortality in girls 3 years post vaccination, suspected to be linked to the higher post vaccination antibody concentrations following this high dose vaccine [90]. Taken together, one would wonder if some precision vaccinology whereby vaccines with different antigen concentrations are used in male versus female children

such that females receive lower dose vaccines may be of benefit to optimize the use of the extremely useful measles vaccine. This may be a solution for vaccine shortage situations where fractional doses could be considered in girls. A similar dose modification strategy was recently successfully implemented for flu vaccines for the elderly [91]. The use of such varied dose interventions would however be a subject for further studies.

As with yellow fever vaccine lower pre-vaccination concentrations for measles correlated with lower post vaccination responses but better seroconversion. We have also previously documented this for MenAfriVac as discussed above [51]. The consistency of this finding for different antigens suggests that lower antibody concentrations at the point of vaccination correlate with lower post vaccination concentration. It is however easier to then attain a 4-fold increase in post vaccination titres which is one of the measures of vaccine immunogenicity. This may not however correlate with attainment of antibodies above a defined threshold and suggests that one parameter in isolation may not be sufficient to comment on vaccine immunogenicity. For antigens like measles where a definite correlate of protection exists, this may be less relevant than for antigens where there is no definite correlate of protection.

Six months post vaccination, there was as expected a slight decline in IgG antibody concentrations. Similar trends for association as with the 28-day post vaccination time point were noted at 6 months. Of note, 30 (2.7%) of the 1,105 infants no longer had antibodies above the definite threshold defined for protection compared to only 2 children at 28 days post vaccination. This finding supports the recommendation for booster vaccination in infants [37] as these concentrations are likely to wane further over time.

Limitations:

As with the data for yellow fever responses, most infants in this study (77%) received the measles vaccine at 9 months of age. There were also relatively few malnourished children. Thus, comparisons of the effect of nutrition would benefit from further exploration in larger studies, sufficiently powered to test such specific co-variables.

5.3 Conclusions

In conclusion, yellow fever seroconversion rates were significantly lower in Ghanaian children compared to infants in Mali. They were unaffected by season of vaccination, sex or nutritional status, although there was a trend to higher titres in males and in children with higher height for age z-scores, which is a measure of chronic malnutrition. There may also be a relationship between the response to natural exposure and nutritional status.

Given the low seroconversion rates in Ghana in particular and the implications for protection at both the personal and population level, our data support the requirement for further analyses of

existing data and samples from young infants in different regions in order to be confident that the WHO recommendation of a single vaccine dose of yellow fever vaccine is indeed providing the intended level of protection for life at both individual and population level. This is further supported by the longitudinal anti-yellow fever antibody titre data which we present below. Such studies should also include longitudinal follow up studies to measure longevity of responses. There may be further need to systematically examine differences between the yellow fever vaccine strains contained in the variety of yellow fever vaccine preparations to guide best use of the available vaccines by decision makers.

Seroconversion rates to measles vaccine on the other hand were not significantly impacted by anthropometric measures of malnutrition. This is a reassuring finding. Of note, virtually all children in this cohort had pre- vaccination IgG antibody concentrations below the definite threshold for protection implying that infants at this age are vulnerable to infection and may support the call for earlier measles vaccine doses. In addition, the observed sex differences would benefit from further studies to understand the factors mediating these differences and assess their clinical relevance- if any. Understanding the dynamics mediating these differences would help tailor immunization programmes to the population and could potentially inform future vaccine development should precision vaccinology for boys versus girls be considered. By 6 months post-vaccination, a small proportion no longer had protective immunity. This supports the need for booster vaccines.

6 Longevity of response to yellow fever vaccine

6.1 Background

There is ongoing debate regarding the longevity of response to a single yellow fever vaccine and this has been identified as a priority research area by WHO, particularly in special groups such as infants, immunocompromised and individuals who received fractional doses of the vaccine [58, 59, 62]. Expanding the body of knowledge in this area is crucial to guiding future policy on the use of the vaccine. This is particularly important as the current WHO recommendation of a single vaccine dose for life may leave millions vulnerable to infection particularly in endemic areas, should they not mount an appreciable response, or the persistence of protection be limited.

The current evidence on antibody longevity following vaccination is varied. A study from Brazil reports only 69% of children had protective titres 10 years post vaccination [85, 92]. In Dutch adults however it was demonstrated that following 1 dose of yellow fever vaccine, over 98% of adults retained protective titres up to 10 years post vaccination following a fractional dose [93].

This, contrasts with 82% of US adults retaining protective titre following 1 standard dose of yellow fever vaccine [94]. There is however a paucity of data from children which is important especially in endemic settings where the single dose for life would be given in infancy when responses may not be the same as for adults.

6.2 Rationale and methods

The low seroconversion rate for yellow fever vaccine response in Ghana calls into question the longevity of protection in the children in that cohort. This is particularly so, because antibody responses to some vaccines including those to yellow fever vaccine tend to dip after an initial rise. The initial peak is often maximal at around 28 days in the immediate post vaccination period and drops of within the first 6 months to 1 year with a subsequent plateau [95, 96]. Thus, the antibody concentrations may be even lower in this cohort following a period of time as the responses reported above should be at about the point of maximal responses. Samples from the same Ghanaian and Malian cohort described at 28 days post vaccination time point above were not available to assess longevity of response. Samples for an extended time point for the Ghanaian cohort are however currently being collected by another team.

In order to address the important question of longevity of protection, I assayed yellow fever antibody responses in another cohort of African children from Gambia and Mali for whom banked serum samples were available. This separate cohort has been described above in section 3.1.1 – Study pers-002-003. The assays were done in the same laboratory as the assays for the Malian and Ghanaian cohorts reported above, using similar methods as follows. Titres of neutralizing antibodies to yellow fever virus were determined by a microneutralization assay. To do this, 100 TCDI50 infectious doses of a yellow fever virus suspension (strain YF-213, Robert Koch Institute) were reacted with serial two-fold dilutions of sera before inoculation into Vero cells cultured in 96-well plates. The cells were microscopically examined for cytopathic effect after seven days. Reference serum samples were run with each plate to ensure suitability of assay and minimize batch effects.

Baseline titres and baseline anthropometry were not available for correlation with these titres.

The age at vaccination was unavailable. These children had all been vaccinated as part of routine EPI programmes prior to enrolment in the study. Prior vaccination with all EPI vaccines due by 12 months of age which include yellow fever vaccine was a prerequisite for trial enrolment. Prior vaccination was confirmed from immunization records but not correlated with a date of birth which would have enabled calculation of age at receipt of yellow fever vaccine as this outcome was not determined for the initial study.

6.3 Results

This cohort included 481 children of which 53.4% were males. The mean antibody titres to yellow fever vaccine by microneutralization five to six years post vaccination in children who had received a single dose of yellow fever vaccine within routine EPI programmes prior to 12 to 23 months of age was 26.7 {95% CI (21.7 – 31.7)}. The median antibody titre was 12.0 {interquartile range (4.0 – 22.0)}. Of note, 21.8% of the cohort had undetectable antibody titres at this time point, with another 5% revealing concentrations below the defined titre for protection of 1:5. Antibody titres by country are summarized in Table 6.1.

The antibody titres did not differ significantly by country (p = 0.09) although there was a trend to higher titres in the Malian cohort.

Table 6.1: Persistence of anti-yellow fever antibody titres 5 – 6 years post vaccination

	Mean (95% CI)	Median (IQR)	Percentage with undetectable antibody (%)	Percentage with antibody below protective threshold (%)
Overall (N = 481)	26.7 (21.7 – 31.7)	12.0 (4.0 – 22.0)	21.8	26.8
Mali (N =238)	30.5 (21.5 – 39.5)	12.0 (0.0 – 22.0)	25.6	28.6
Gambia (N = 243)	23.0 (18.3 - 27.7)	12.0 (5.0 – 22.0)	19.7	25.1

95% CI = 95% confidence interval
IQR = interquartile range
YF Ab = yellow fever antibody
N = number of observations
NB: Percentage with titres below the protective threshold includes those with undetectable titres

The distribution of titres is represented in figure 6.1 revealing non-statistically significantly higher titres in Mali and showing the generally low antibody tires in these children 5 to 6 years post vaccination with a single dose of yellow fever vaccine.

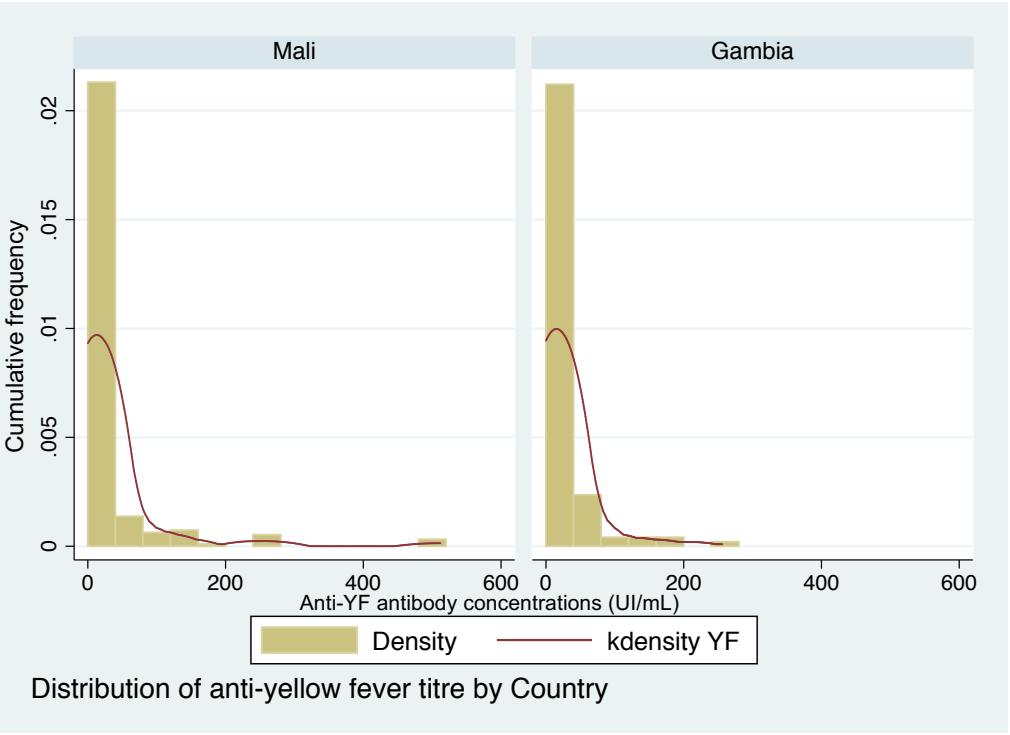


Figure 6.1 Distribution if anti-yellow fever antibody titre by Country
Kernel density: non-parametric estimation of probability density function of anti-yellow fever titre
NB: P value for difference in antibody titre between countries = 0.1401

Sex, country and time since vaccination did not affect the longevity of antibody titre in uni- or multivariable regression models for antibody titre as a continuous variable (Table 6.2)

Table 6.2 Association of covariates at time of vaccination with log2 anti-yellow fever antibody titres 5 – 6 years post vaccination

		Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**Coef. (95% CI)**	**p-value**	**Coef. (95% CI)**	**p-value**
Sex					
male*	256	0.00 - -	-	0.00 - -	-
female	224	1.94 (-8.17 to 12.06)	0.7061	0.03 (-0.17 to 0.03)	0.7407
Season					
dry*	311	0.00 - -	-	1.00 - -	-
rainy	170	-3.27 (-13.8 to 7.27)	0.5425	-0.05 (-0.27 to 0.17)	0.6570
Country					
Mali*	232	0.00 - -	-	0.00 - -	-
Gambia	179	-7.56 (-17.61 -2.49	0.1401	-0.13 (-0.34 to 0.08)	0.2160
Time since vaccination	-	-0.04 (-1.30 -1.21)	0.9477	0.19 (-1.73 to 2.11)	0.8475

NB: Age and sex missing for 1 participant

** Reference stratum*

N = number of observations; Coef = coeficient; 95% CI = 95% confidence interval

Results of separate uni- and multi-variable linear regression

Multi-variable model adjusted for, age, sex and season of vaccination and time since vaccination

The odds of maintaining an antibody titre above the defined threshold of protection of 1:5 was also unaffected by the sex, season of vaccination or time since vaccination (Table 6.3).

Table 6.3 Association of covariates at time of vaccination with maintaining a threshold of anti-yellow fever antibody titres of 1:5 or more 5 – 6 years post vaccination

			Univariable (crude)		Multivariable (adjusted)	
Covariate	**N**	**mean**	**OR ((95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Sex						
male*	256	25.8	1.00 - -	-	1.00 - -	-
female	224	27.8	0.97 (0.65 to 1.45)	0.8689	0.97 (0.65 to 1.45)	0.8806
Season						
dry*	311	27.9	1.00 - -	-	1.00 - -	-
rainy	170	24.6	0.86 (0.56 to 1.30)	0.4634	0.83 (0.54 to 1.27)	0.3943
Country						
Mali*	232	30.5	1.00 - -	-	1.00 - -	-
Gambia	179	23.0	1.21 (0.81 to 1.81)	0.3611	1.17 (0.78 to 1.76)	0.4497
Time since vaccination	-	-	0.99 (0.94 to 1.04)	0.7898	0.96 (0.00 to 1.27)	0.3943

NB: Age and sex missing for 1 participant

** Reference stratum*

N = number of observations; Coef = coeficient; 95% CI = 95% confidence interval

Results of separate uni- and multi-variable logistic regression

Multi-variable model adjusted for, age, sex and season of vaccination and time since vaccination

6.4 Discussion

Our data suggest that over 25% of infants who received a single dose of yellow fever vaccine as part of routine EPI schedules are unprotected by 5 – 6 years after vaccination defined by correlate of protection of titres of 1:5. This worrying finding has significant implications for defining vaccination policies for infants who received yellow fever vaccine as part of routine EPI programmes in view of WHO's current recommendations of a single vaccine dose for life. Recent studies following fractional dosing of yellow fever vaccine to Dutch adults demonstrated that up to 10 years post vaccination 98% of the individuals vaccinated were still protected [93]. The sample size was however small (40 individuals) and represented only 48% of the original recipients of the vaccine [93]. While the findings following a fractional dose of yellow fever vaccine differ from ours, where a full dose of vaccine was administered, longevity following fractional doses may be worse than that following a full dose. However, vaccines were administered to

different age groups: – adult vs infant and different settings – non-endemic Europe versus endemic Africa. These factors may also explain difference, given the entirely different natural exposure between non-endemic and the endemic settings and potentially between age groups. A study reporting antibody persistence in US adults following a full dose of yellow fever vaccine reports that all individuals who received multiple doses of yellow fever vaccine had protective titres irrespective of the time since previous vaccination. Of individuals who had received only 1 dose however, if that dose was less than 10 years from the time of testing, 94% of them had protective titres. When 10 or more years had elapsed however, only 82% had protective titres [94]. This study does therefore suggest that even in adults booster vaccinations may still be warranted in certain populations, as argued in a recent opinion piece by a prominent vaccinologist [85]. There is however a paucity of data on persistence of immunity to yellow fever in children and thus our findings are important and timely and will inform the discussions around future vaccination policy for yellow fever vaccine. Published evidence does suggest that only 69% of children in Brazil maintained protective titres 10 years after receiving a full vaccine dose [85, 92]. These articles however do not state the vaccine used which may be an important consideration for the longevity of vaccine response.

The sex, season of vaccination and time since vaccination did not impact the longevity of anti-yellow fever antibody titre 5 - 6 years post vaccination in our study. This is similar to findings from antibody persistence studies 3 to 4 years post vaccination in a cohort of adult travellers vaccinated with yellow fever vaccine in the US. Early onset of viraemia and higher antibody titre one-month post vaccination were the only factors associated with higher antibody titres 3 to 4 years post vaccination [95]. Such variables (onset of viraemia and antibody titres one-month post vaccination) were not available for our analysis.

Conclusion

There is an urgent need to consolidate the evidence around the potential need for booster vaccination with yellow fever vaccine following full and fractional doses in children and adults, and this priority has been identified by WHO [59, 62, 97]. This question is even more urgent to answer as fractional doses are recommended in the face of outbreaks where there are yellow fever vaccine shortages [62].

Our findings would suggest that children in sub-Saharan Africa may require booster doses at least by school entry age.

6.5 Future plans related to longevity of response to yellow fever

Ultimately, samples will be sought from the regions with low versus high responders to compare long term responses and assess functionality more closely in studies designed to assess this

outcome. In addition, I aim to seek funding to conduct adequately powered sero surveys for yellow fever antibody titres from a cross section of the population to include school age children to further inform vaccination policy. This will hopefully form part of my post-doctoral studies.

7. Correlating serum nutritional parameters to vaccine response (current findings informing future plans)

7.1 Background and Rationale:

Serum nutritional parameters with known immune modulating effects such as iron, vitamin D, may also modulate the response to vaccines. These effects of iron and vitamin D status are poorly studied in humans [98-100].

Available data suggest the highest vitamin D deficiency is found in Asia, Africa and the Middle East [99]. Vitamin D is documented to influence innate and T cell immunity and shift balance towards a Th1-type immune response [100]. There is also recent evidence to support a link between iron and vitamin D with a potential additive effect [99, 100].

Maternal iron deficiency is well documented in low and middle-income countries (LMIC) and has been shown to affect new born iron status [98, 101, 102], Iron also influences innate immune responses, including the activation of NF-κB, a transcription factor, essential for the expression of several genes involved in innate immunity and inflammation [98, 102]. Nutritionally deprived mice have been shown to sequester iron from immune cells thus impairing immune function [102].

For both iron and vitamin D, supplementation is potentially available, and improved understanding of their impact on immunity in general and vaccine responses in particular could inform evidence-based use of supplements for mothers or infants and/or use of currently available vaccines or aid new vaccine development.

7.2 Methods:

My work in the past 3 years has included co-leading a clinical study to characterize the ontogeny of molecular development in new born infants [103]. This involved a pilot study recruiting 30 newborn infants between November and December 2015, within 24 hours of life from whom a blood sample was collected at this time point. A second sample was collected at day 1, 3 or 7 of life from 10 infants each [103]. Each 2ml sample was used to assess the transcriptome, metabolome and proteome of each newborn at 2 time points with the first week of life [103]. The findings from the proteomic and transcriptomic portions of the analysis relate to this thesis.

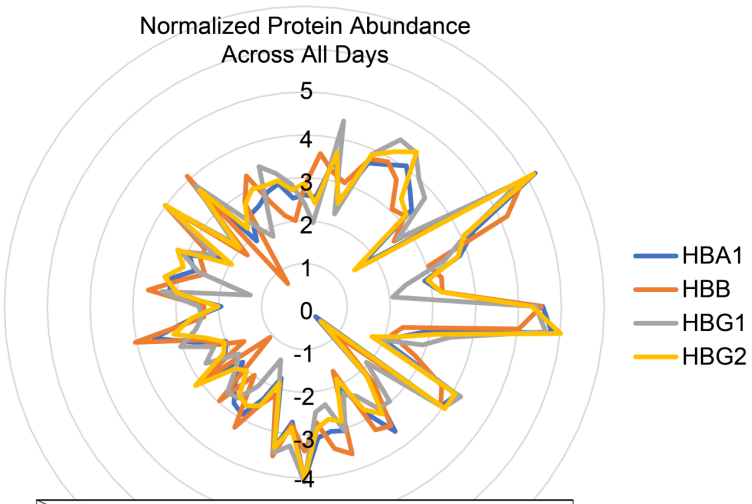
The proteomic assessment was done by mass spectrometry. The samples were grouped according to the day of life, and proteins that were not quantifiable in at least five of the samples in any day were filtered out [103]. Proteins which showed statistically significant differences in abundance between the days of life were identified using a paired t test.

For the transcriptomic assessment, total ribonucleic acid (RNA) was extracted from the sample using the RiboPure RNA purification tool. Strand-specific cDNA libraries were generated from oly-adenylated RNA using the KAPA stranded RNA-Seq library preparation kit. All cDNA libraries were generated from poly-adenylated RNA. Genes with very low counts and globin transcripts were filtered out. Paired analysis with Wald statistic tests were used to identify differentially expressed genes. A pairwise correlation of plasma concentrations of haemoglobin (Hb) subunits -alpha (HBA1), -beta (HBB), -gamma-1 (HBG1), and -gamma-2 (HBG2) was also performed for each sample [103].

7.3 Results:

A portion of the results from this large multi-site effort relates to my PhD studies as presented below.

A striking finding related to a nutritional marker, was the upregulation of scavenging heme – a product of erythrocyte breakdown, from plasma in addition to upregulation of the complement cascade which is also important for innate immune responses. This upregulation of heme scavenging may be similar to the phenomenon previously reported in nutritional deprived mice [102] and may also impair immune function in these infants. We also found that haemoglobin was abundant in the samples. The pairwise correlation of the different Hb subunits revealed that while the concentration of Hb was sample specific the ratio of the different haemoglobin subunits was similar between the samples and conserved over the first week of life. These varying Hb concentrations may be correlated with maternal Hb and there is some evidence that maternal Hb impacts the new-born Hb [104].



p-value / R	HBA1	HBB	*HBG1*	HBG2
HBA1		5.0E-20	1.9E-15	2.2E-39
HBB	0.8773		1.4E-08	1.6E-15
HBG1	0.8169	0.6577		1.5E-15
HBG2	0.9748	0.8205	0.8178	

Figure 7.1 Normalized abundance of haemoglobin subunits in newborn serum.

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The transcriptomic analysis also detected dramatic developmental changes when comparing day of life 0 to days 3 and 7. A number of pathways including cellular responses to stress, detoxification of reactive oxygen, and iron uptake and heme biosynthesis were down regulated. Other genes were up regulated including those involved in complement activation and interferon signaling [103].

7.4 Rationale for planned future analysis

The upregulation of heme scavenging and Hb findings in this pilot study have sparked my interest in understanding the potential impact of iron/heme regulation at the maternal/newborn interface on subsequent responses to vaccines given at birth in the sub Saharan African context. In this context, deficiency of iron – a known immune modulator - is a major factor and the impact of the regulation of this known immune modulator on the response to vaccination during a period of documented iron deficiency and significant changes in iron regulation is key. Maternal iron deficiency is well documented in low and middle-income countries (LMIC) and has been shown to affect newborn iron status [98]. Iron also influences innate immune responses, including the activation of NF-κB, a transcription factor, essential for the expression of several genes involved in innate immunity and inflammation [102]. Nutritionally deprived mice have been shown to sequester needed iron from immune cells thus impairing immune function [102].

I have identified vitamin D as an additional immune modulator whose impact on vaccine immunogenicity is also under researched. Available data suggest the highest vitamin D deficiency is found in Asia, Africa and the Middle East. Vitamin D is documented to impair innate and T cell immunity and shift balance to a Th1 response [99, 100]. There is also recent evidence to support a link between iron and vitamin D which may result in an additive effect [105].

7.5 Planned methodology for follow up:

To test the hypothesis that these immune modulators may modulate the response to infant vaccines, I plan to correlate markers of serum iron (serum iron, C reactive protein, transferrin, soluble transferrin receptor, ferritin, and hepcidin), and vitamin D metabolism (25(OH)D and 3-epi-25(OH)D concentration) from mothers and infants to innate and vaccine induced immune responses. This will form part of my post-doctoral research and will be conducted within ongoing studies recruiting mother-infant pairs at the Medical Research Council Unit The Gambia at London School of Hygiene and Tropical Medicine. I will examine the relationship between maternal iron and vitamin D status and subsequent infant response to BCG and Hepatitis B vaccines, vaccines administered soon after birth in low- and middle-income countries (LMIC). I will also relate these vaccine responses to the infant iron and Vitamin D status and conduct additional exploratory proteomic analyses on infant samples to gain comprehensive insights.

7.5.1 Research Questions

1. Does maternal iron status influence innate immune responses in newborns?
2. Does maternal iron status influence the immune response to BCG and Hepatitis B vaccines in newborns?
3. Can differences in infant immune responses be explained by the iron status of the mother and /or iron and vitamin D status of the infant or additional proteomic parameters?

In ongoing studies, blood samples are being collected from women delivering at term at major health facilities in The Gambia and their infants at birth and following BCG and Hep B vaccination. Iron and vitamin D status will be measured in maternal samples; infant samples will be used to measure innate and vaccine-specific immune responses, the proteome, iron and vitamin D status.

Innate responses will be measured using cytokine and chemokine assays and TLR stimulation of whole blood; BCG responses will be measured using a flow-based cell mediated immunity (CMI) assay; Hepatitis B responses will be measured by IgG ELISA and CMI during the first week of life, and at ages 30 and 128 days. Iron status will be assessed using specific

iron indicators, inflammatory markers and hepcidin. Mass spectrometry will be used to quantify 25 hydroxy vitamin D (25(OH)D) concentration and 3-epi-hydroxyvitamin D (3-epi-25(OH)D) - measures of vitamin D metabolism.

8. Overall Conclusions:

Seroconversion rates following yellow fever vaccination were significantly lower in Ghanaian compared to Malian infants. They were unaffected by season of vaccination, sex or nutritional status, although there was a trend to higher titres in males and in children with higher height for age z-scores, which is a measure of chronic malnutrition.

There may be further need to systematically examine differences between the yellow fever vaccine strains contained in the variety of yellow fever vaccine preparations to guide best use of the available vaccines by decision makers. This is informed by the fact that receiving vaccines of varying compositions was the only major difference identified between these cohorts. Reassuringly, seroconversion rates to measles vaccine were not significantly impacted by anthropometric measures of malnutrition. Virtually all children in this cohort however had pre- vaccination IgG antibody concentrations below the definite threshold for protection implying that infants at this age are vulnerable to infection possibly supporting the call for earlier measles vaccine doses. This factor is particularly key given the recent spate of measles outbreaks worldwide. In addition, the observed sex differences (higher responses in females) would benefit from further studies to understand the factors mediating these differences and assess their clinical relevance- if any. Understanding the dynamics mediating these differences would help tailor immunization programmes to the population and could potentially inform future vaccine development should precision vaccinology relating to vaccine doses for boys versus girls be considered. By 6 months post-vaccination, a small proportion no longer had protective immunity supporting the need for booster measles vaccines.

Anti-yellow fever antibody titres 5 – 6 years post vaccination were below protective thresholds in 26.8 % of infants studied and completely undetectable in 21.8 %. This suggests an urgent need to consolidate the evidence around the potential need for booster vaccination with yellow fever vaccine following full and fractional doses in children and adults. This question is even more urgent to answer as fractional doses are recommended in the face of outbreaks where there are yellow fever vaccine shortages. Our findings would suggest that children in sub-Saharan Africa may require booster doses at least by school entry age which is at variance with WHOs current policy of a single vaccine dose for life.

Other nutritional factors such as iron status are known immune modulators and have been shown in our recent work to be down regulated in the immediate post delivery period. This may

have impact for responses to vaccines given during this period of rapid development after birth given the immune modulatory properties and will be the focus of my immediate post-doctoral research.

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10. Appendices

Statement on Pre-release and Contribution

The analysis of the relationship between yellow fever vaccination titres and anthropometry has been accepted for publication in 'Expert Review of Vaccines'. This manuscript was written by the PhD candidate. The analysis was designed and conducted by the candidate with support from Prof Beate Kampmann and Dr Elmar Saathoff.

The portion on iron status of the newborn has been published (see *Nature Communications* manuscript below) and further analysis will be the basis of future work.

Other portions of the thesis on measles vaccine responses and the longevity of the response to yellow fever vaccine are currently being prepared for submission for peer review and subsequent publication.

Manuscripts attached to monographic thesis**:**

See section 1, 2, 3, 4and 5 of thesis

1. **Idoko OT,** Mohammed N, Ansah P, Hodgson A, Tapia M, Sow S, Chowdhury PR, Niedrig M, Saathoff E, Kampmann B, *Antibody responses to yellow fever vaccine in 9 – 11-month-old Malian and Ghanaian children*. Expert Review of Vaccines. 2019 doi.org/10.1080/14760584.2019.1640118.

Candidates Contribution:

This manuscript outlines the IgG responses to yellow fever vaccines measured by sero neutralization assay in 9 to 11-month-old (at time of vaccination) African infants from Ghana and Mali. Neutralizing antibodies following vaccination were unaffected by season of vaccination, sex, pre vaccination titres or nutritional status measured by anthropometry. There was however a trend for higher titres in males and children with higher z scores for age (a measure of chronic malnutrition). The seroconversion rates differed significantly between the 2 countries studied suggesting a possible need for booster vaccinations.

The candidate designed and conducted the analysis for this manuscript and wrote the manuscript.

See section 8 of thesis

2. Lee AH*, Shannon CP*, Amenyogbe N*, Bennike TB*, Diary-Arce J*, **Idoko OT*,** Gill EE, Ben-Othman R, Pomat WS, van Haren SD, le Cao KA, Cox M, Darboe A, Falsafi R, Ferrari D, Harbeson DJ, He D, Bing C, Hinshaw SJ, Ndure J, Njie-

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NB: * Joint co first authors

Candidates contribution:
This manuscript outlines changes at a molecular level to a newborn within the first 7 days of life a period crucial for immune development which ultimately impacts the response to vaccines. Using multi omic techniques the data reveal a dynamic purposeful molecular trajectory of development over the first week of life. Of note we demonstrated upregulation of home scavenging during the first week of life. There is evidence to suggest that this may be an attempt to protect the infant from infection by making iron less available for microorganisms (an immune defence mechanism). Given the prevalence of iron deficiency in sub-Saharan Africa where I live and work, especially during the crucial period of pregnancy, I intend to take this finding into future studies to explore the impact of iron markers and metabolism on the innate and vaccine responses at the maternal-newborn interphase. Details of this are given in Section 8 of the thesis.
I designed the clinical protocols and documents used to undertake the study and recruited all 30 newborn infants whose data and samples were assessed. I also provided clinical oversight and wrote the clinical methods section of the manuscript. I contributed to data interpretation and reviewed the full manuscript.

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j. And my Lord and God in whom all things hold together.