Modeling the long-term persistence of hepatitis A antibody after a two dose vaccination schedule in Argentinean children

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ABSTRACT

Background: Long-term seroprotection data are essential for decision-making on the need and timing of vaccine boosters. Based on data from longitudinal serological studies, modeling can provide estimates on long-term antibody persistence and inform such decision-making.

Methods: We examined long-term anti-hepatitis A virus (anti-HAV) antibody persistence in Argentinean children ≤15 years after the initial study where they completed a two-dose course of inactivated hepatitis A vaccine (Avaxim® 80U Pediatric, Sanofi Pasteur, Lyon, France). Blood serum samples were taken at baseline, 2 weeks (post first dose), 6 months (pre-booster), 6.5 months (post-booster), 10 years and 14-15 years after first vaccine dose. We fitted eight statistical model types, predominantly mixed effects models, to anti-HAV persistence data, to identify the most appropriate and best fitting models for our dataset and to predict individuals' anti-HAV levels and seroprotection rates up to 30 years post vaccination.

Results: Fifty-four children (mean age at enrolment 30.4 months) were enrolled up to 15 years post first vaccine dose. There were three distinct periods of antibody concentration: rapid rise up to peak concentration post-booster, rapid decay from post-booster to 10 years, followed by slower decay. A three-segmented linear mixed effects model was the most appropriate for the dataset. Extrapolating based on the available 14-15 year follow-up, the analysis predicted that 88% of individuals anti-HAV seronegative prior to vaccination would remain seroprotected at 30 years post vaccination and lifelong seroprotection for vaccinees seropositive prior to vaccination.

Conclusion: Currently available data demonstrate that Avaxim® 80U Pediatric confers to most vaccinees a high level of seroprotection against hepatitis A infection for at least 20-30 years.

INTRODUCTION

Hepatitis A virus (HAV) infection is the most common cause of acute viral hepatitis [1], causing an estimated 119-126 million acute hepatitis cases and 34,000-35,245 deaths worldwide in 2005, according to independent estimates. Infection with HAV induces lifelong immunity and can be measured by the concentration of antibodies against HAV (anti-HAV). Amongst older children and adults, seroprevalence is in general extremely high in low-income regions such as sub-Saharan Africa and very low in high-income regions such as Western Europe and North America, but increases have been observed between 1990 and 2005 [1]. Improved standards of sanitation and water supply in lower-income regions have decreased transmission of HAV. With less HAV circulating within populations, the average age of infection is driven upwards. This can have the paradoxical effect of increasing HAV-related mortality and morbidity, because disease severity is strongly age dependent, with adults far more likely to develop clinical illness [2, 3]. Therefore HAV vaccination may play a key role in preventing increases in HAV-related disease in regions with changing patterns of HAV transmission.

Both inactivated and live attenuated HAV vaccines have been developed. Both these vaccine types are highly immunogenic and immunization will generate long-lasting, possibly life-long, protection against hepatitis A in children as well as in adults [4]. There do not yet exist clinical trials of HAV vaccines of sufficiently long duration to empirically demonstrate life-long persistence of anti-HAV antibodies, although longer-term data are beginning to become available [5, 6]. Therefore many studies have attempted to estimate long-term persistence based on short-term data using various modeling methods for hepatitis A [7-9] and for other vaccine preventable infections including diphtheria,[10, 11] hepatitis B,[12] meningitis A,[13] pertussis,[14] Japanese encephalitis,[15], and HPV,[16] in order to address duration of vaccine protection. Such models generally employed either exponential-type or linear

modelling approaches according to whether antibody titres were in natural units or log-transformed. While earlier models estimated antibody persistence at a population level, there has been a trend toward individual-level modeling in more recent publications.

Argentina has been classified as being of intermediate-level HAV endemicity [1], but there is evidence of decreasing seroprevalence among younger age groups in the last 15 years [17, 18]. Long-term anti-hepatitis A virus antibody persistence has been examined in Argentinean children 10 years after the initial study in which they received two doses of inactivated hepatitis A vaccine (Avaxim® 80U Pediatric, Sanofi Pasteur, Lyon, France) [19, 20]. In the 10-year follow-up study we found that the vaccine conferred long-term protection amongst children who were seronegative prior to vaccination.

Data are now available for this cohort up to 14-15 years post first HAV vaccination (primary vaccination of two-dose protocol). We have used these new data to investigate by extrapolation the long-term anti-HAV antibody persistence for: a) the mean duration of seroprotection from HAV infection conferred by the Avaxim® 80U Pediatric vaccine; and b) the percentage of seropositive vaccinees at 20, 25 and 30 years post vaccination. We have employed a range of statistical models to investigate the models which most appropriately fit the anti-HAV antibody persistence data.

MATERIALS AND METHODS

Trial Design and Study Population

Of 537 healthy Argentinean children aged 12 months to 15 years enrolled between December 1996 and January 1997 from a single study centre (Hospital de Niños "Dr. Ricardo Gutiérrez", Buenos Aires, Argentina) in an open, non-controlled trial of inactivated Avaxim®

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80U Pediatric vaccine (Sanofi Pasteur, Lyon, France), 54 children aged 12 to 60 months were invited for serum anti-HAV measurements at 10 years post first vaccine dose, from May to November 2007 (89% seronegative pre-vaccination) (Figure 1). The full vaccination course administered in 1996-7 consisted of two doses administered six months apart. Only subjects completing the course were selected for follow-up. Of this cohort, 33 were additionally followed at 14-15 years post first dose (91% seronegative pre-vaccination), between December 2010 and February 2012. Pre-defined exclusion criteria were: 1) having received an additional booster dose of hepatitis A vaccine after the second dose; 2) having had moderate or severe illness (such as varicella) or immunodeficiency; and 3) having had previous treatment with growth hormone or human immunoglobulins or having received whole blood cells or blood product transfusion during the previous six months. This was to ensure blood samples collected would provide reliable measures of anti-HAV concentrations. However, no subject was excluded from taking part in the study based on any of these criteria. The trial protocol was approved by the internal review board and ethics committee of the Hospital de Niños "Dr. Ricardo Gutiérrez". Further information on the cohort has been previously published [19, 20].

One patient with anti-HAV concentration measurements from all six time points was missing date of follow-up for the 2 week (post-first dose visit), 6 month (pre-booster) and 6.5 month (post-booster) time points. These dates were imputed using the median time to each of these visits for the rest of the cohort.

Laboratory Tests

Serum anti-HAV antibody concentrations were measured by VIDAS Anti-HAV Total (HAVT, BioMerieux, France). The assay combines a two-step enzyme immunoassay competition method with fluorescent detection (ELFA) [21]. Results are expressed in

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mIU/mL (WHO reference standard first Reference Preparation Hepatitis A Immunoglobulin [100 mIU/mL]). Sera with anti-HAV ≥20 mIU/mL (lower limit of quantification, LLOQ) were considered seropositive. At the time of current study the serological test-system used in the initial study (antibody concentrations had been assessed using commercial radioimmunoassay [HAVAB, Abbot Laboratories, North Chicago] modified to increase the sensitivity) was not available [21].

Statistical Analysis

We investigated different approaches used previously to analyze antibody persistence following vaccination [9, 10, 12, 14-16, 18, 22-27], in order to identify the most appropriate and best fitting models for our dataset. Previous analyses of anti-HAV decline post vaccination have employed extrapolation methods assuming exponential decay [7-9] and so we have investigated this approach (Models 1 and 2). There is also a large body of literature on antibody decay which adopts mixed effects models for similarly shaped antibody decay curves. Mixed effects models contain both fixed and random effects, which are particularly appropriate for fitting to such longitudinal data and allowing for the dependence of within-child measurements [28, 29]. We have therefore employed mixed effects models as the underlying structure of all other models evaluated (Models 3 to 8). We additionally investigated the impact of adding the covariates age, gender and anti-HAV serostatus at enrolment to these models.

Models 1 and 2 are extrapolations based on methods previously used to estimate duration of seroprotection of hepatitis A vaccines [7-9]. Model 1 extrapolates using geometric mean concentrations (GMCs) taken at peak concentration (post-booster) and 14-15 years post vaccination and assumes exponential decay of $\log_{10}$ antibody over time, so Y_t, $\log_{10}$ antibody at time t, is defined as:

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$$Y_t = Y_{0.5} 10^{-\delta(t-0.5)}$$

where $Y_{0.5}$ is $\log_{10}$ antibody at time 0.5 years (representing the post-booster time point) and δ is the decay coefficient (rate of antibody decline). Rearranging gives:

$$\delta = \frac{\log_{10}(Y_{0.5}) - \log_{10}(Y_t)}{t - 0.5}$$

for $t > 0.5$ years post baseline. Using GMCs:

$$\delta = \frac{\log_{10}(GMC_{0.5}) - \log_{10}(GMC_{14})}{\bar{t}_{14} - \bar{t}_{0.5}}$$

where $GMC_{0.5}$ and GMC_{14} are the GMCs (of the $\log_{10}$ antibody concentrations) at the post-booster and 14-15 year time points respectively. $\bar{t}_{14}$ and $\bar{t}_{0.5}$ represent the average time of the 14-15 year and 0.5 year time point antibody concentration measurements respectively, for all study participants (because time of each concentration measurement varied between study participants).

Model 2 is extrapolation based on individual data rather than GMCs, following a similar method to Wiens et al [8] and Van Damme et al [7]:

$$\delta = \frac{\log_{10}(Y_{0.5}) - \log_{10}(Y_{14})}{t_{14} - t_{0.5}}$$

using the same notation as Model 1. The rate of antibody decline is the geometric mean of all individually calculated values of δ. The limitations of the extrapolation approach are the lack of any indication of variance of the results, such as confidence intervals (Model 1) and the inability to incorporate covariates or assess goodness of fit using Akaike's Information Criterion (AIC) [30] and the Bayesian Information Criterion (BIC) [31] statistics (both models).

Model 3 is a linear mixed effects model involving linear antibody decay containing fixed and random effects for both slope and intercept parameters: $Y_{ij} = (a + a_i) + (b + b_i)t_j + \varepsilon_{ij}$

where Y_{ij} is $\log_{10}$ antibody concentration for subject i observed at time t_j, a and a_i are the population-level (fixed effect) and individual-level (random effect) intercepts and b and b_i are the population-level and individual-level slope corresponding to the rate of linear antibody decay. ε_{ij} is the residual error between model prediction and the observed value. The model was fitted to: a) 6 month, 10 year and 14-15 year data; and b) 10 year and 14-15 year data only i.e. model was only fitted to the antibody decline phase (from peak antibody concentration at 6 months post vaccination onwards).

Model 4 is an exponential-type mixed effects model constructed from peak antibody concentration (fitted to 6 month, 10 year and 14-15 year measures) with fixed and random effects for slope $(a+a_i)$, intercept $(b+b_i)$ and exponent (c) parameters:

$$Y_{ij} = (a + a_i) + \sum_c (b + b_i)t_j^c + \varepsilon_{ij}$$

where c can be up to 4 (i.e., models can include quadratic, cubic and up to power 4 terms of the time covariate).

Model 5 is a segmented linear mixed effects model which contains fixed and random effects for both slope and intercept parameters (as for Model 3) but additionally allows fitting to antibody concentration measurements from all six time points:

$$Y_{ij} = (a + a_i) + (b + b_i)t_{ij}\,\delta_{ij} + (f + f_i)t_{ij}\left(1 - \delta_{ij}\right) + \varepsilon_{ij}$$

where δ_{ij} is an indicator: $\delta_{ij}=1$ for time up to six months (i.e. up to peak antibody concentration) and $\delta_{ij}=0$ for time post six months period of antibody decline. Model 6 is similarly segmented but additionally involves exponent parameters (c) as described for Model 4. We fitted a linear model for the antibody increase phase i.e. up to 6 months follow-up, then an exponential model for the antibody decline phase (post six months).

$$Y_{ij} = (a + a_i) + (b + b_i)t_{ij}\,\delta_{ij} + \sum_c (f + f_i)t_{ij}^c\left(1 - \delta_{ij}\right) + \varepsilon_{ij}$$

Models 7 and 8 are only fitted to data for the antibody decline phase (post-booster time point onwards) but informed by the initial rate of antibody increase. This involves including an additional fixed effect covariate representing the slope from the linear trend from the first four time points (i.e. up to 6 months). Model 7 is a linear mixed effects model of the form:

$$Y_{ij} = (a + a_i) + (b + b_i)t_{ij} + g_j + \varepsilon_{ij}$$

where t_j represents time points post 6 months i.e. antibody decline phase only, f_i is the coefficient for g_i that represents the b_i term from Model 5 i.e. the coefficient of the slope of antibody increase for t_j time points up to six months. Model 8 is an exponential-type mixed effects model and so additionally involves exponent parameters:

$$Y_{ij} = (a + a_i) + \sum_c (b + b_i)t_{ij}^c + g_j + \varepsilon_{ij}$$

g_i represents the b_i term from Model 6.

We compared the fit of each model to the dataset to identify the most appropriate model to use to predict duration of seroprotection post vaccination. AIC and BIC were calculated for Models 3 to 8 but cannot be calculated for the extrapolation methods used for Models 1 and 2. In general, models with smaller AIC and/or BIC values indicate a better model fit to the dataset; however they are not appropriate for comparisons of models constructed using different sample sizes. We used the AIC/BIC statistics where suitable plus visual assessment and evaluation drawing on our experience regarding biological plausibility, to evaluate how well the predicted values reflected the observed values of the dataset, in order to identify the most appropriate model to use to predict duration of seroprotection of the vaccine.

The best fitting model was used to predict individuals' antibody concentrations and seroprotection up to 25 years post vaccination, as well as the corresponding proportion of seroprotected individuals and the mean duration of protection after two doses of Avaxim®

80U Pediatric vaccine. All models used unconstrained variance-covariance matrices. All analyses are based on antibody concentrations on a $\log_{10}$ scale. Statistical significance was defined as $p<0.05$. Models were constructed and fitted using Stata 12 (StataCorp LP).

To predict the duration of seroprotection of the vaccine requires estimation of the HAV seroprotection threshold. Currently, there is no clear definition of this for anti-HAV following vaccination. Thresholds of 10 mIU/mL [32], 15 mIU/mL [33] and 20 mIU/mL [9] have been used previously, and generally the LLOQ of the assay used is considered to be the protective level [34]. Anti-HAV concentrations of 20 mIU/mL after administration of immunoglobulins are known to protect again HAV infection [35, 36]. We have therefore used for this analysis a conservative estimate of 20 mIU/mL conferring protection.

RESULTS

Cohort characteristics

Between December 2010 and February 2012, 33 children were followed up at 14-15 years post first vaccination. The remaining 21 children of the cohort were followed up at 10 years only, May to November 2007 and then lost to follow-up (Figure 1). Of these 54 children with long-term follow-up, 27 (50%) were male; mean age at enrolment was 30.4 months (standard deviation [SD] 12.5, range 11.6-59.9 months). There was no statistically significant difference in age between seropositives and seronegatives (27.6 months [SD 19.9] and 30.7 months [SD 11.6], respectively (p=0.717). Two subjects who were followed to 14-15 years and were seronegative pre first vaccination reported receiving immunosuppressive therapy during the preceding six months: one received aerosol prophylaxis for asthma twice weekly (Simbicort™), while the other received Flutivent™, one puff daily, also aerosol asthma prophylaxis. However, as asthma prophylaxis was not considered to prevent the development of vaccine-induced anti-HAV antibodies, these subjects were not excluded from analyses.

This is consistent with summary of product characteristics which show that immune system disorders reported with these products are uncommon to rare.[37, 38]

Antibody concentrations over time

Figure 2 shows the antibody concentrations of patients at each time point. It reveals three distinct periods of antibody concentration: a rapid rise from baseline up to the period post vaccine booster administration (GMC 5920 mIU/mL, 95%CI 4758-7364, subjects seronegative at enrolment); relatively rapid decay post peak to 10 years follow up (to GMC 261 mIU/mL, 95%CI 199-341), followed by slower decay between the 10 year and 14-15 year follow up periods (to GMC 253 mIU/mL, 95%CI 181-353). In a minority of subjects, anti-HAV concentrations increase by the 14-15 year time point (Figure 2, seropositive children plot). Children HAV-seropositive prior to vaccination appear to reach higher peak concentrations and have a slower rate of antibody decline post-booster, but the small sample size (n=6) means trends must be interpreted with caution. One subject had become seronegative by the 10 year concentration measurement but was lost to follow-up at year 14-15. At 14-15 years, 100% of the subjects were found to have seropositive anti-HAV concentrations.

Model selection

Table 1 provides parameter estimates and fit statistics for each modeling approach. Models 1 and 2 gave a reasonable fit to the observed data using eyeball assessment but no formal evaluation of fit was possible because the AIC and BIC statistics could not be calculated. Furthermore, these methods could not allow for the incorporation of covariates (see Pigeon et al for a discussion of limitations to this approach [39]). The inclusion of covariates age at enrolment and gender in each of Models 3 to 8 did not improve goodness of fit, while

inclusion of anti-HAV serostatus prior to vaccination did, resulting for some models, in a coefficient which was statistically significant (data not shown). Therefore serostatus was retained in the models but age and gender were dropped from the final model selected for each model type.

Model 3 using all three time points of antibody decline (post-booster, 10 and 14-15 years) demonstrated a statistically significant yearly decrease in antibody concentration of 0.117 $\log_{10}$ mIU/mL per year (p<0.001, Table 1). Restricting Model 3 to using the 10 year and 14-15 year time points only, in order to reflect the slower antibody decline phase characteristic of individuals after one year post peak antibody concentration, generated lower AIC/BIC statistics but this does not indicate better model fit because the models are using different patient numbers. Time since vaccination and serostatus pre-vaccination were no longer significant in the two time point model (p=0.163, p=0.133 respectively, Table 1) because the antibody decline by this time period has flattened out (Figure 2). This implies that from 10 years onwards, anti-HAV levels are not dependent on time since vaccination and are stable.

Model 4 encompasses a range of models which add time as a covariate, exponentiated by increasing order terms. Adding a quadratic term for time since vaccination resulted in a statistically significant coefficient (0.009, p<0.001, Table 1). Since this coefficient is positive, the model would predict when projected over time that antibody levels would start to increase, as the influence of the quadratic (positive) term outweighs the linear (negative) term . Model 4 order 2 (quadratic term) has an improved fit compared to Model 3 using three time points (AIC 88, BIC 111, Table 1) but the positive quadratic term implies an infinite duration of protection, as antibody levels are ultimately predicted to increase over time. Additionally including cubic and quadratic terms for time since second vaccine dose into the model did not

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improve model fit and these terms were not statistically significant (cubic model AIC 90, BIC 116, Table 1; results for fourth order model not shown).

Models 5 and 6 are segmented, which enables fitting to concentrations from all six time points, with a change point at the post-booster peak concentration time point, separating the phases of antibody decrease and decline. This model type captures the initial increase trend and can predict concentrations at all times post first vaccination, not just post-booster (as for Models 1 to 4). The linear mixed effects segmented model (Model 5) demonstrates a significant increasing antibody trend pre-vaccine booster (1.83 mIU/mL/year p<0.001, Table 1) and decreasing trend post-booster (-1.94 mIU/mL/year p<0.001). However, the AIC and BIC values suggest a poor model fit to the data. This is due to the high variability of pre-booster concentrations (Figure 2) and the necessary inclusion of more covariates in the model (which determine the segmented structure).

Model 6, involving a quadratic time since vaccination term for the antibody decline segment only, provided a better fit to the data and all covariates remained significant (Table 1).

Models 7 and 8, fitting to the anti-HAV decline phase but including initial rate of anti-HAV increase as a covariate, did not meaningfully improve fit to data above that of models using the decline phase data only (Models 3 and 4).

Quadratic models appear to better capture the decreasing trend of $\log_{10}$ antibody concentration, but the quadratic terms in Models 4, 6 and 8 are all positive (Table 1) and so these models would all predict eventual rises in anti-HAV concentrations, because of the shape of the quadratic function, which is biologically implausible. Therefore, we have chosen as the best model the segmented linear mixed effects model with three segments (i.e., two changing points, Model 5), reflecting the three phases of concentration level: increase to post-booster time point, relatively rapid decline to 10 year time point; stable concentration 10

to 14-15 years. Figures 3 and 4 show the individual predicted plots (as a spaghetti plot and trellis graphs, respectively) of anti-HAV against observed values, demonstrating an adequate fit to the data. The majority of subjects' observed and fitted values demonstrate a stable or declining trend between 10 and 14-15 years, while a few subjects (6 out of 33 with 14-15 year data) showed a slight increase in anti-HAV concentrations.

Table 2 shows the observed anti-HAV concentrations at 10 and 14-15 year time points and those predicted by our chosen model for 20, 25 and 30 years post first vaccine dose, stratified by anti-HAV serostatus of children prior to vaccination. It also shows the percentage of children seroprotected at each time point. Levels of anti-HAV decrease very slowly over time, as levels plateau after 10 years post first vaccination (Figure 2). Children seropositive prior to vaccination, through natural exposure to hepatitis A, demonstrate 100% seroprotection up to 30 years post first vaccine dose. Seroprotection rates are slightly lower for children seronegative prior to vaccination, with 96%, 96% and 88% who are predicted to remain seroprotected at 20, 25 and 30 years post first vaccine dose. The predicted mean concentration of anti-HAV at years 20, 25 and 30 years are 208, 181, 156 mIU/mL amongst children seronegative prior to vaccination. Predicted mean concentration of anti-HAV at years 20, 25 and 30 years for children seropositive prior to vaccination are 387, 335, 290 mIU/mL. Reported values of anti-HAV concentrations at 20, 25 and 30 years are extrapolations based on the available 15 year follow-up data.

DISCUSSION

On the basis of the data available, the most suitable model, based on statistical criteria, biological plausibility and beliefs regarding patterns of antibody decay over time, was the linear segmented mixed effects model (Model 5). This model was similar to previous analyses that assumed linear decay in log units for the long-term phase of the antibody decay

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trend, which is the most relevant phase for prediction of antibody persistence, and also in the adoption of fixed and random effects [14, 15]. However, this model additionally includes data from the early phase of antibody rise post first-dose, thus maximizing utilization of the dataset.

The best fitting models in terms of AIC and BIC goodness of fit statistics included quadratic terms, but such models fail for long-term projections in terms of biological plausibility, because we would not expect anti-HAV concentrations to increase over time (in the absence of natural boosting). In their discussion of the appropriate modeling approach, Bailleux et al state that in general, non-linear approaches are applicable to the full follow-up period whereas a linear approach is, "appropriate applied to the period after the initial rapid decay phase stabilizes" [14]. In the absence of additional follow-up time points between the peak post-booster measure and 10 years' follow-up, it was most appropriate to focus on fit to the plateau phase rather than model the full period of antibody decline, as we had sparse data in the rapid decay phase. The segmented models allow full use of the dataset, by incorporating information from early time points as well as those from the anti-HAV decline period.

Our analysis predicts that the seroprotection rate after two doses of Avaxim® 80U Pediatric vaccine remains high (88%) for at least 30 years after first vaccination. A full vaccination course consisting of two vaccine doses confers long-term immunity from HAV infection by the induction of persistent vaccine-induced anti-HAV antibodies.

Age at vaccination and gender were not found to be significantly associated with pattern of antibody decline, but serostatus prior to vaccination was significant. Children who were anti-HAV seropositive before first hepatitis A vaccination exhibited slower rates of antibody decline and are predicted to remain seropositive to at least 30 years post vaccination. These

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children may have natural hepatitis A infection-mediated in addition to vaccine-mediated immunity.

This study is one of the longest duration follow-ups post hepatitis A vaccination in children. Van Herck et al have reported the 17 year follow-up of health adults after two dose inactivated hepatitis A vaccine [5]. Raczniak et al recently reported anti-HAV levels amongst subjects who were vaccinated with inactivated hepatitis A vaccine as children, 17 years previously [40], but this was a cross-sectional study of a convenience sample. Thus to our knowledge, the current study is the longest prospective study of children post hepatitis A vaccination.

There are some limitations to this study. With no antibody concentration measurements between the post-booster sample (at 6 months post baseline) until the 10 year measurement, we cannot estimate the change point at which the phase of rapid antibody decline ends and the slower decay phase begins. In the absence of sufficient data to estimate this point, the segmented models have used the point of peak antibody concentration (post-booster measure) and 10 year follow-up point as change points, but it is more likely that the second change point, the point at which rapid antibody decline is replaced by a more stable phase, is around 6-12 months post-booster, as has been observed in other antibody persistence studies [15, 33, 41]. Another important limitation is the relatively small sample size which decreases further with increasing follow-up. Two subjects received immunosuppressive therapy at the 14-15 year time point (aerosol asthma prophylaxis). However, as noted previously these subjects were not excluded from analyses which benefited sample size. Exclusion of these subjects would have further diminished the limited sample size at this time point (n=33).

Our predictions required us to extrapolate data beyond the 14-15 year period of observation, which implicitly assumes that the linear rate of antibody decay must continue to the time

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horizon of our prediction. Indeed a few subjects did demonstrate an increase in the anti-HAV concentration between 10 and 14-15 years counter to the trend in the majority of subjects, possibly due to natural exposure to HAV. However, based on our model comparisons, the linear assumption would appear justified and is consistent with antibody persistence studies for other diseases.[10, 14, 15] However, any extrapolation of statistical models outside the period of observation should be interpreted with caution.

In our analysis, we conservatively adopted a threshold of ≥20 mIU/mL to represent protection from HAV infection. However, previous studies of vaccine-mediated HAV immunity have demonstrated that as well as inducing seroprotective antibodies, even a single dose can induce cellular immunity, and an immunologic memory response to booster vaccination some years later, thus providing protection even in the absence of detectable HAV antibody concentrations [4, 35, 42-46]. In another anti-HAV persistence study in Argentina, Espul et al found that among six children seronegative (antibody concentration <10 mIU/mL) during follow-up after a single dose of Avaxim® 80U Pediatric vaccine, all had strong responses to a vaccine booster dose, suggesting that the duration of protection provided by vaccination may be longer than suggested by the decline in antibody level [32]. Thus the duration of protection conferred by two dose Avaxim® 80U Pediatric may be even longer than can be estimated based on anti-HAV concentrations alone. Furthermore, the plateauing of anti-HAV levels between 10 and 14-15 years means that the definition of anti-HAV level conferring seroprotection is essentially moot, because our chosen model predicts such slow antibody decline. The other group of models that fitted the data relatively well involved a quadratic term, which would even have predicted an increase in anti-HAV levels over time, not a decrease.

Our study findings of stable antibody levels up to 15 years post vaccination support the findings of other studies and suggest durability of the anti-HAV immune response after

vaccination. We cannot exclude the possibility that in this setting, anti-HAV concentrations remained stable because of natural exposure of study participants to HAV being transmitted in the population (considerable exposure to wild-type hepatitis A virus has been observed in another recent childhood vaccination study in Argentina [32]). However, Raczniak et al found that, with a three-dose schedule of inactivated hepatitis A vaccine, anti-HAV levels remained above the seroprotection threshold at 17 years post vaccination and had plateaued in the previous seven years [40]. The study was conducted in the US and so natural boosting would have been less likely than in our study from Argentina. Therefore we can say with more confidence that inactivated hepatitis A vaccines confer long-term (beyond 15 years) protection from HAV infection. Longer duration follow-up is required to predict total duration of this protection with more confidence, as the plateauing of antibody levels observed in our study and by Raczniak et al [40] imply lifelong immunity, but we cannot exclude the possibility of another change point leading to antibody decline after longer duration post-vaccination.

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FIGURES LEGENDS

Figure 1 Study profile.

Figure 2 Double box plot figure of hepatitis A antibody concentration by time point and serostatus of children at baseline. The central line of each box is the geometric mean concentration; upper and lower hinges of the box are geometric mean 95% confidence intervals. Adjacent lines represent maximum and minimum values. The dashed line represents the threshold of protection for hepatitis A, conservatively estimated as 20 mIU/mL.

Figure 3 Spaghetti plot of the predicted and observed anti-HAV antibody concentration values for children seronegative at baseline, up to 15 years post vaccination. Fitted lines use the segmented mixed effects linear model with three segments: antibody increase up to peak post-booster; relatively rapid decrease to 10 years; slow decrease to 14-15 years.

Figure 4 Trellis plot showing predicted and observed anti-HAV antibody concentration values for children seronegative or seropositive at baseline, up to 15 years post vaccination. Fitted lines use the segmented mixed effects linear model with three segments: antibody increase up to peak post-booster; relatively rapid decrease to 10 years; slow decrease to 14-15 years.

Table 1 Parameter estimates and fit statistics for each modeling approach.

Model	Anti-HAV parameters	Mean parameter estimates (95%CI)	AIC	BIC
1	Slope $(-\delta)$	-0.101^{2}	N/A[4]	N/A[4]
2	Slope $(-\delta)$	-0.097 $(-0.086,-0.109)^{3}$	N/A[4]	N/A[4]
3	*Three time points:*			
	Intercept $(a + a_i)$	3.71 (3.48,3.95)	144	167
	Slope $(b + b_i)$ – year	-0.117 $(-0.126,-0.107)$ p<0.001		
	Seropositive at enrolment	0.488 (0.159,0.816)		
	Two time points:			
	Intercept $(a + a_i)$	2.51 (2.34,2.66)	60	75
	Slope $(b + b_i)$ – year	-0.125 $(-0.030,0.005)$ p=0.163		
	Seropositive at enrolment	0.281 $(-0.085,0.647)$ p=0.133		
4	*Quadratic model:*			
	Intercept $(a + a_i)$	3.14 (3.03,3.25)	88	111
	$(b + b_i)$ – year	-0.171 $(-0.183,-0.158)$ p<0.001		
	$(b + b_i)^{2}$ – year^{2}	0.009 (0.007,0.011) p<0.001		
	Seropositive at enrolment	0.491 (0.179,0.803) p=0.002		
	Cubic model:			
	Intercept $(a + a_i)$	3.31 (2.42,4.20)	90	116
	$(b + b_i)$ – year	-0.158 $(-0.223,-0.094)$ p<0.001		
	$(b + b_i)^{2}$ – year^{2}	-0.002 $(-0.056,0.053)$ p=0.957		
	$(b + b_i)^{3}$ – year^{3}	0.001 $(-0.003,0.005)$ p=0.703		
	Seropositive at enrolment	0.492 (0.180,0.804) p=0.002		
5	*Two segments:*		503	536
	Intercept $(a + a_i)$	1.39 (1.24,1.54)		
	$(b + b_i)$ – year, increase phase	1.83 (1.49,2.16) p<0.001		
	$(f + f_i)$ – year, decline phase	-1.94 $(-2.28,-1.61)$ p<0.001		
	Seropositive at enrolment	0.845 (0.475,1.216) p<0.001		
	Three segments:			
	Intercept $(a + a_i)$	1.39 (1.24,1.54)	489	529
	$(b + b_i)$ – year, increase phase	1.84 (1.51,2.16) p<0.001		
	$(f + f_i)$ – year, decline phase 1	2.53 $(-5.96,11.02)$ p=0.559		
	$(g + g_i)$ – year, decline phase 2	-4.37 $(-12.85,4.11)$ p=0.313		
	Seropositive at enrolment	0.820 (0.450,1.190) p<0.001		
6	Intercept $(a + a_i)$	1.39 (1.24,1.54)	480	502
	$(b + b_i)$ – year, increase phase	1.83 (1.51,2.16) p<0.001		
	$(f + f_i)$ – year, decline phase	-2.08 $(-2.41,-1.75)$ p<0.001		
	$(f + f_i)^{2}$ – year^{2} decline phase	0.010 (0.005,0.014) p<0.001		
	Seropositive at enrolment	0.820 (0.450,1.191) p<0.001		
7	Intercept $(a + a_i)$	3.71 (3.48,3.95)	144	167
	$(b + b_i)$ – year, increase phase	-0.117 $(-0.126,-0.107)$ p<0.001		
	g_i – rate of antibody increase pre-booster	0.0219 $(-0.0360,0.0797)$ p=0.458		
	Seropositive at enrolment	0.488 (0.159,0.816) p=0.004		
8	Intercept $(a + a_i)$	3.81 (3.58,4.04)	90	116
	$(b + b_i)$ – year, increase phase	-0.240 $(-0.264,-0.216)$ p<0.001		
	$(b + b_i)^{2}$ – year^{2}	0.00918 (0.00748,0.01088) p<0.001		
	g_i – rate of antibody increase pre-booster	0.0273 $(-0.0289,0.0835)$ p=0.341		
	Seropositive at enrolment	0.529 (0.207,0.851) p=0.001		

AIC - Akaike's Information Criterion; anti-HAV – anti-hepatitis A antibody; BIC – Bayesian

Information Criterion, 95%CI – 95% confidence interval.

1 Assuming 20 mIU/mL confers protection from HAV infection.

2 No confidence or credible interval produced using Model 1.

3 Geometric mean and 95% confidence interval used for Model 2 for comparability with

Model 1.

4 AIC and BIC goodness of fit statistics cannot be calculated for Models 1 and 2.

Table 2 Observed and predicted seroprotection rate, by serostatus at enrolment. Predictions based on the segmented mixed effects linear model with three segments.

Time post first vaccine dose	Seronegative at enrolment		Seropositive at enrolment	
	Anti-HAV GMC, mIU/mL (95%CI)	x/n, % seroprotected[1] (95%CI[2])	Anti-HAV GMC, mIU/mL (95%CI)	x/n, % seroprotected[1] (95%CI[2])
10 years (observed)	261 (199-341)	47/48, 97.9% (87.5,99.9)	587 (101-3401)[3]	6/6, 100.0% (51.7,100.0)
14-15 years (observed)	253 (181-353)	30/30, 100.0% (85.8,100.0)	779 (1-874,238)[3]	3/3, 100.0% (31.0,100.0)
20 years (predicted)	208 (128-340)	46/48, 95.8% (84.6,99.3)	387 (157-953)	6/6, 100.0% (51.7,100.0)
25 years (predicted)	181 (93-351)	46/48, 95.8% (84.6,99.3)	335 (122-922)	6/6, 100.0% (51.7,100.0)
30 years (predicted)	156 (67-367)	42/48, 87.5% (74.1,94.8)	290 (92-915)	6/6, 100.0% (51.7,100.0)

GMC – geometric mean concentration; HAV – hepatitis A virus; n – total number of patients followed up; x - number of patients followed up who were seropositive at that time point.

1 Seroprotection defined as anti-HAV concentration ≥20 mIU/mL.

2 95%CIs calculated using the Wilson method with continuity correction (45, 46).

3 There are wider 95%CIs for observed than predicted time points, as predicted values are derived from the linear segmented mixed effects model, which draws on information from all subjects i.e., seronegative as well as seropositive at enrolment. Observed values are from only six (10 year time point) and three (14-15 year time point) subjects for the group seropositive at enrolment.
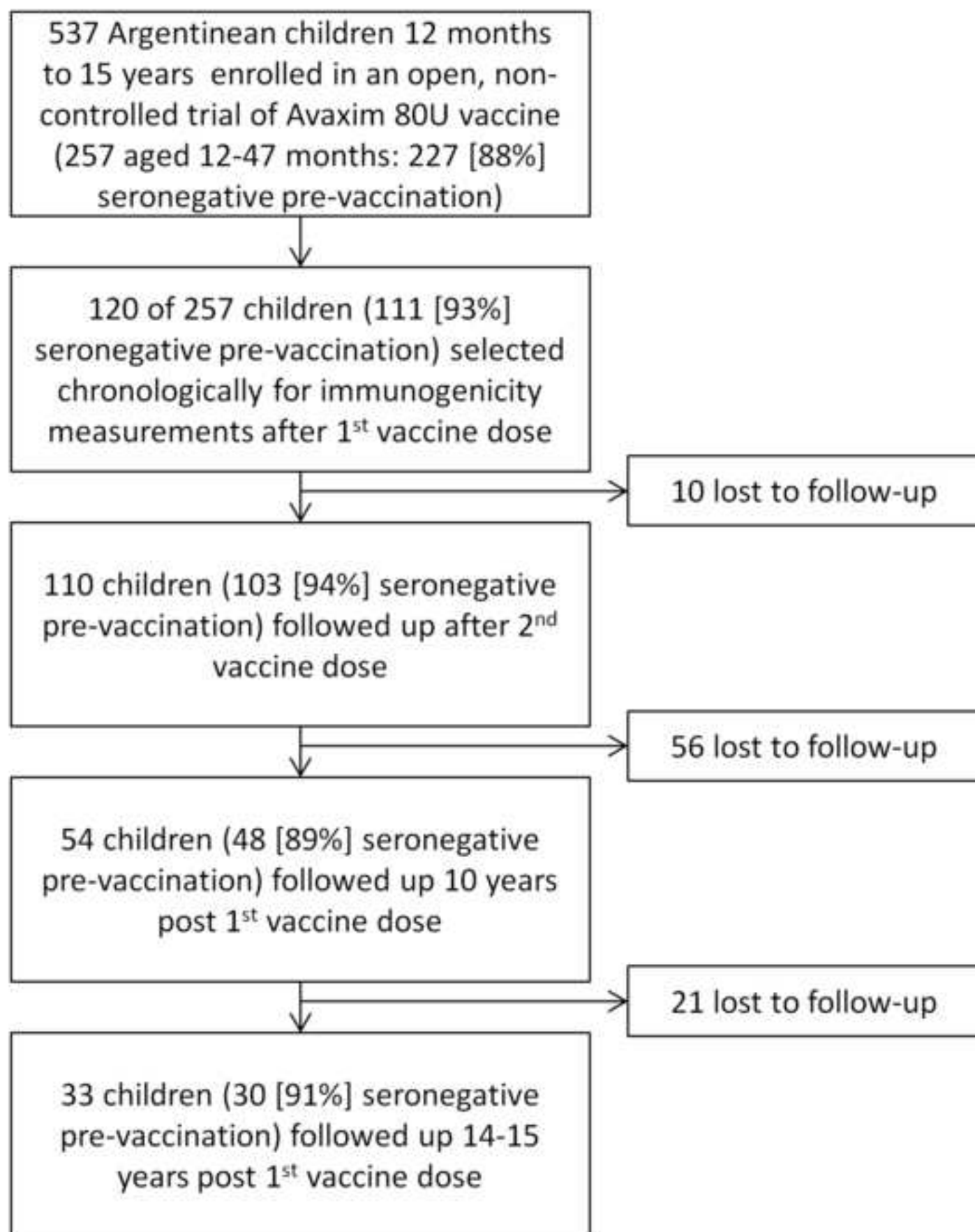
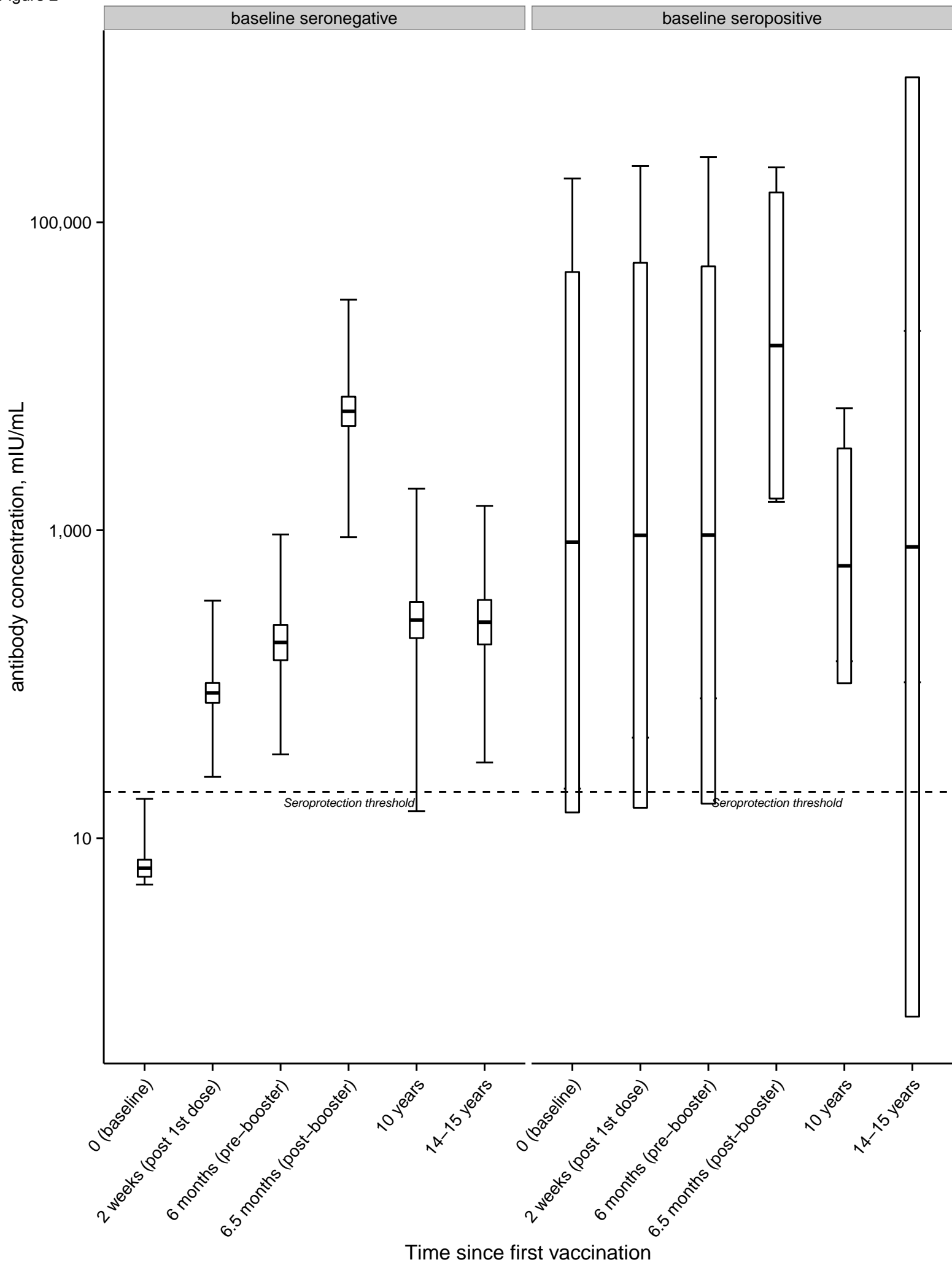Figure 1
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Figure 1

537 Argentinean children 12 months to 15 years enrolled in an open, non-controlled trial of Avaxim 80U vaccine (257 aged 12-47 months: 227 [88%] seronegative pre-vaccination)

120 of 257 children (111 [93%] seronegative pre-vaccination) selected chronologically for immunogenicity measurements after 1st vaccine dose

10 lost to follow-up

110 children (103 [94%] seronegative pre-vaccination) followed up after 2nd vaccine dose

56 lost to follow-up

54 children (48 [89%] seronegative pre-vaccination) followed up 10 years post 1st vaccine dose

21 lost to follow-up

33 children (30 [91%] seronegative pre-vaccination) followed up 14-15 years post 1st vaccine dose

Figure 2
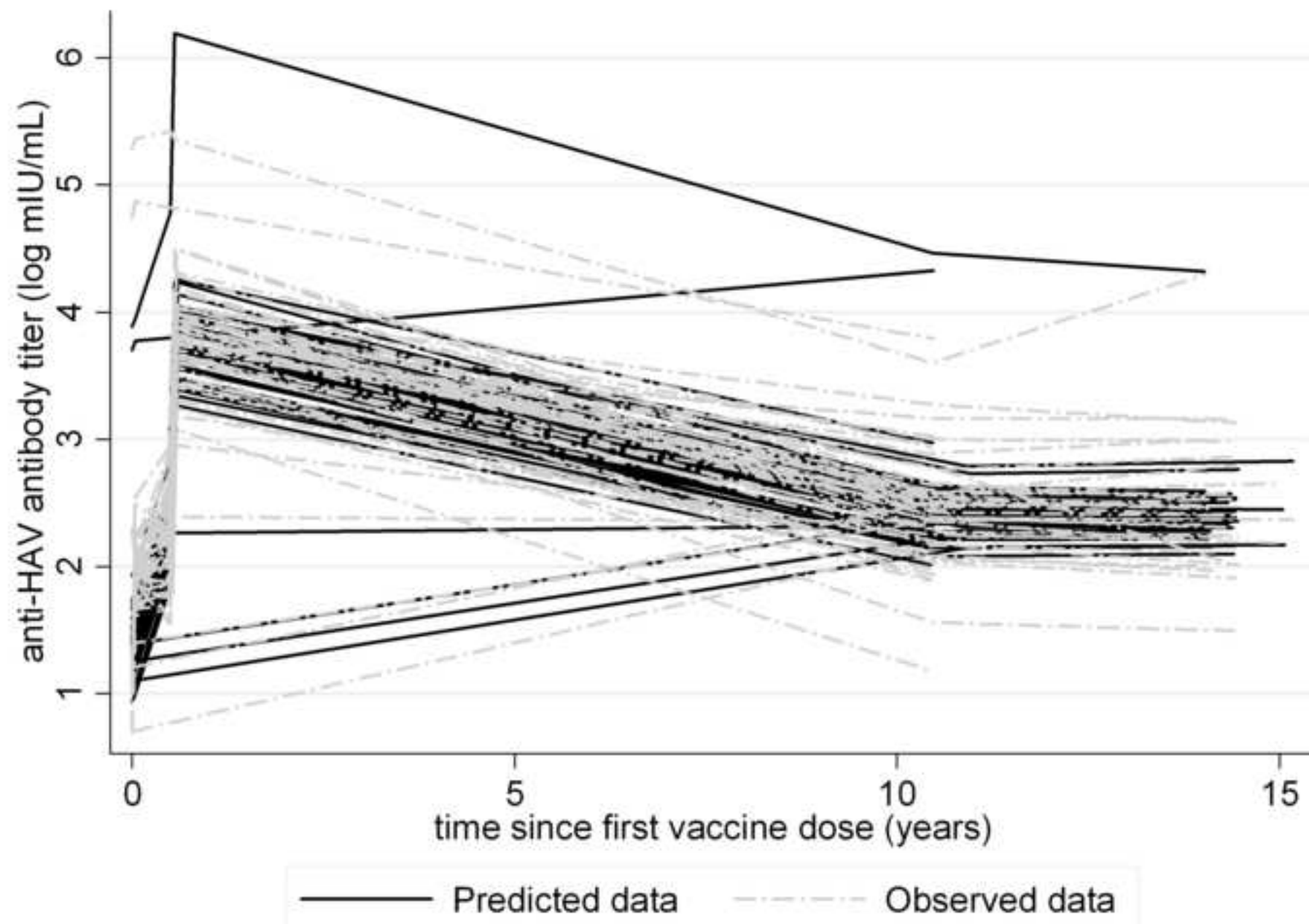
Figure 3
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anti-HAV antibody concentration (mIU/mL)

time since first vaccine dose (years)