MODELING A FIELD TRIAL OF THE RTS,S/AS02A MALARIA VACCINE

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Abstract. A double-blind, phase IIb, randomized controlled trial of the malaria vaccine RTS,S/AS02A showed an efficacy of 45.0% in reducing the force of infection for Plasmodium falciparum and of 29.9% in reducing incidence of clinical malaria in children 1–4 years of age in Manhiça, Mozambique. We simulate this trial using a stochastic model of P. falciparum epidemiology, and the setting-specific seasonal pattern of entomologic inoculations as input. The simulated incidence curve for the control group was comparable with that observed in the trial. To reproduce the observed efficacy in extending time to first infection, the model needed to assume an efficacy of 52% in reducing the force of infection. This bias arises as a result of acquired partial immunity against blood stages, thus suggesting an explanation for the lower observed efficacy in a previous trial in semi-immune adult men in The Gambia. The shape of the incidence of infection curve for the vaccine cohort in Manhiça indicates that the vaccine provides incomplete protection to a large proportion of the vaccinees, rather than offering complete protection to some recipients and none to others. This behavior is compatible with a model of no decay in efficacy over the six-month surveillance period of the trial. The model accurately reproduced the lower efficacy against clinical disease than against infection. In the simulations this finding resulted from loss of acquired clinical immunity as a result of a reduction in the force of infection in the vaccinated cohort. The model also predicted greater efficacy against severe diseases than against clinical disease. The success of the simulation model in reproducing the results of the Manhiça trial encourages us to apply the same model to predict the potential public health and economic impact if RTS,S/AS02A were to be introduced into the existing expanded program on immunization.

INTRODUCTION

A double-blind, phase IIb, randomized controlled trial in Manhiça district, Mozambique, found an estimated 45.0% efficacy of the malaria vaccine RTS,S/AS02A for delaying the time to first Plasmodium falciparum infection in children 1–4 years of age. The vaccine efficacy for delaying time to first clinical episodes was 29.9%. In a previous trial carried out with semi-immune adult men in The Gambia, vaccine efficacy for extending time to first infection was 34%. Time to first observed infection is a different outcome from the true proportion of infections prevented by RTS,S vaccination, and there are a number of reasons why these trial estimates of efficacy are likely to be lower. They may be diluted by effects of heterogeneity in vaccine effect between individuals. Pre-existing naturally acquired pre-erythrocytic immunity could also introduce variations between hosts in susceptibility to infection, while asexual blood stage immunity means that some hosts are more likely to appear to be parasite negative, even though they are infected. Such heterogeneities consistently lead to reduction in estimates of efficacy in preventing infection.

The different efficacy values all have wide confidence intervals (Table 1), and can therefore be reconciled with a statistical null hypothesis that the variation between them represents only a result of chance. However there are good reasons for expecting malaria vaccines to have different efficacies against different outcomes. Acquired immunity is potentially an even more important source of bias in analyses of post-infection outcomes such as incidence of clinical disease, than in those of infection. Post-infection outcomes can also potentially be a cause of attenuated efficacy estimates.

The latter is particularly an issue with definitions of clinical malaria episodes used in vaccine trials, which are known to have imperfect specificity.

Many of these phenomena lend themselves to simulation-based analyses. We now simulate the Manhiça trial. Our main objective was to test a stochastic model of the primary epidemiologic effect of RTS,S/AS02A. The model makes use of models of malaria incidence, parasite densities, and of clinical malaria, to predict the outcomes of the trial. This enabled us to consider the extent to which the trial results can be explained by established features of P. falciparum epidemiology in the Manhiça setting. The results also suggest that the model could provide a basis for predicting the potential effects of future malaria vaccine trials elsewhere, and for estimating their potential public health and economic impact.

MATERIALS AND METHODS

Study site and trial procedures. The trial was carried out between April, 2003 and May 2004 among 2,022 children 1–4 years of age living in Manhiça district, Mozambique. Children were randomly allocated three doses of either the pre-erythrocytic malaria vaccine RTS,S/AS02A or control vaccines. The primary endpoint was time to first clinical malaria episode of P. falciparum, defined as axillary temperature ≥37.5°C plus P. falciparum asexual parasite density > 2,500 per µL of blood. Surveillance of this endpoint was in a cohort of 1,605 children recruited from the area around Manhiça itself (cohort I) who were monitored over a six-month period after completion of the vaccination schedule. Vaccine efficacy for prevention of new infections was determined in cohort II, which was composed of 417 children recruited in the area of Ilha Josina located north of Manhiça.

Entomologic inoculation rate. The monthly average entomologic inoculation rate (EIR) was determined for the period of the trial only for the zone around Manhiça where cohort I...
TABLE 1
Efficacy estimates for children 1–4 years of age*

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Manhiça trial†</th>
<th>Simulation (based on 5,400 children per cohort)</th>
</tr>
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<tbody>
<tr>
<td>Prevention of infection</td>
<td>$\overline{V_E}_p$ 0.45 (0.31, 0.56)</td>
<td>$\overline{V_E}_p$ 0.45† (0.42, 0.48)</td>
</tr>
<tr>
<td>Prevention of clinical episodes</td>
<td>$\overline{V_E}_c$ 0.30 (0.11, 0.45)</td>
<td>$\overline{V_E}_c$ 0.33 (0.27, 0.38)</td>
</tr>
<tr>
<td>Prevention of severe malaria</td>
<td>$\overline{V_E}_s$ 0.58 (0.16, 0.81)</td>
<td>$\overline{V_E}_s$ 0.36 (0.26, 0.45)</td>
</tr>
</tbody>
</table>

* Values in parentheses are 95% confidence intervals.
† This value was constrained to be equal to the that of $\overline{V_E}_p$. The remaining efficacy values follow from this constraint, together with the value of 5% for the probability that a malaria fever is treated.

was recruited. Mosquito densities were determined by sampling the households of a random sample of individuals drawn from the demographic surveillance population. The sporozoite rate was determined using a standard enzyme-linked immunoassay technique, and the EIR was estimated by the product of the human biting rate and the sporozoite rate as described for other entomologic surveys in the area. Transmission in the area of Ilha Josina, where cohort II was recruited, is thought to be higher than around Manhiça.

Simulation of P. falciparum incidence and morbidity. We implemented a stochastic simulation model of P. falciparum epidemiology using the monthly pattern of EIR for Manhiça as input to the simulation and making predictions for four simulated cohorts, each of at least 5,000 children 1–4 years of age, the same age-range as in the Mozambique trial. The four cohorts corresponded to the two control and two vaccine cohorts in the actual trial. We used the same computer code, written in FORTRAN, as in our previous simulations.

To model the baseline immunologic status of the trial cohorts, we assumed that from birth until to the beginning of the trial these children had been exposed to the same recurring annual pattern of inoculations that was measured during the trial period, and to the drug regimen for treating malaria described previously for the Manhiça district. For the simulation of the trial, we reproduced the drug regimen described by Alonso and others. To estimate the proportion of clinical episodes that were treated, we compared the recorded incidence in the control group with the incidence predicted by the model. The resulting compliance estimate was then applied uniformly to the simulations of both pre-trial and trial periods. In the absence of setting-specific data on the proportion of severe malaria episodes reporting to the formal health sector, we followed the procedures of Goodman and others and McCombie in assuming that half of the severe malaria episodes report to a health facility and compared the observed hospital admission rate with that predicted by this model.

We considered the model predictions of incidence of first P. falciparum infection, the frequencies and densities of patent parasitemia, the incidence of acute episodes of clinical malaria and the incidence of severe disease. Our simulations followed the original trial protocol in considering only the time at risk for each individual until their first or only episode (analysis of incidence of acute episodes in cohort I), or first recorded patent infection (cohort II).

Simulation of vaccine. The model for simulation of incidence of first P. falciparum infection in the control cohorts is described in an accompanying paper. Briefly, new infections in individual $i$ at time $t$ are modeled via a Poisson process where the force of infection $\lambda(i,t)$ is the product of three independent terms, $E_d(i,t)$, $S(i,t)$, and $S_d(i,t)$, where $E_d(i,t)$ is the EIR, adjusted for the expected size of the host, $S(i,t)$ captures innate density dependent control, and $S_d(i,t)$ measures the effects of acquired immunity. In the absence of vaccination, the function for acquired immunity is

$$S_2(i,t) = \left( S_{imm} + \frac{(1 - S_{imm})}{1 + \left( \frac{X_p(i,t)}{X^*_p} \right) \gamma_p} \right),$$

where $X_p(i,t)$ is the level of naturally acquired pre-erythrocytic exposure measured by the cumulative number of entomologic inoculations, $X^*_p$ is a critical value of the level of exposure, $\gamma_p$ is the steepness of the relationship between exposure and immunity, and $S_{imm}$ determines the survival of inocula in the most highly immune individuals. The fitted curve for $S_2(i,t)$ as a function of $X_p(i,t)$ is given in Figure 1, which shows two alternative modifications of this model for application in the vaccine cohorts.

Model A. Vaccination is equivalent to natural exposure to a cumulative total of $X_p$ entomologic inoculations; thus the function becomes

$$S_2(i,t) = \left( S_{imm} + \frac{(1 - S_{imm})}{1 + \left( \frac{X_p(i,t)}{X^*_p} \right) \gamma_p} \right).$$

**Figure 1.** Functions used to model acquired immunity. Upper line = value of $S_2(i,t)$ fitted to data from Matsari, Nigeria. Dashed line = $S_{2y}(i,t)$ derived from model (a); $X^*_p = 1,800$ inoculations. Lower continuous line = $S_{2y}(i,t)$ derived from model (b); $\overline{V_E}_{0} = 0.52$; EIR = entomologic inoculation rate.
We consider briefly the consequences of model A and conclude that this cannot be distinguished from model B below on the basis of the Manhiça trial data alone.

**Model B.** Vaccination results in reduction in the probability that an inoculum survives by a proportion that we term the underlying vaccine efficacy in preventing infections, \( VE_0(i) \), i.e.,

\[
S_2(i, t) = S_2(i, 0)(1 - VE_0(i))
\]

To allow for possible variation in efficacy between individuals, i.e. the extent to which the vaccine is leaky, we randomly assign values of \( VE_0(i) \) drawn from beta-distributions. We define the underlying overall efficacy, \( VE_0 \), as the mean of \( VE_0(i) \) (Figure 2). A model with a very low value of the parameter \( b \) of the beta distribution corresponds to an all-or-nothing vaccine (i.e., one that offers complete protection to a proportion \( E_0(i) \) of the vaccinated population \( E_0(i) = 1 \) for all \( i \) in the protected group), and no protection at all to the others \( E_0(i) = 0 \), while a high value of \( b \) corresponds to a leaky vaccine.

In both models, we assume that the vaccine efficacy remained constant throughout the surveillance period of the trial. In the main simulations patent parasitemia is assumed to arise at the earliest after a latent period of 15 days (three five-day time units within the simulation).

**Fitting of vaccine efficacy to trial results.** The original analyses of the trial used proportional hazard survival models to obtain empirical estimates, \( \hat{VE}_p \) and \( \hat{VE}_c \) of the overall vaccine efficacy in preventing infections, \( VE_p \), and in preventing clinical attacks, \( VE_c \), respectively. The efficacy in preventing severe malaria was estimated as:

\[
\hat{VE}_s = 1 - P_{sv}/P_{sc},
\]

where \( P_{sv} \) is the proportion of children experiencing severe malaria episodes in the vaccine arm and \( P_{sc} \) is the corresponding proportion in the control arm.

We use the same statistical methods to compute corresponding estimates \( \hat{VE}_p, \hat{VE}_c, \hat{VE}_s \) from our simulated datasets. To determine the best fitting distribution for \( VE_0(i) \) we carried out a search using simulations with different efficacy until \( \hat{VE}_p = \hat{VE}_p \). Since the immune status of each simulated child is known at each time point, we extract summaries of immune status variables to provide explanations of the patterns observed for the efficacy variables.

**RESULTS**

The EIR estimate of 38 inoculations per person per year was higher than that measured in the same area in 1997–1998. Most of the six-month surveillance period in the Mozambique trial fell during the rainy season, with the peak of malaria transmission during the first half of the surveillance (Figure 3). This was reflected in the shape of both observed and predicted infection curves, both of which indicated a very
high initial rate of infection, with a tendency to decrease over time. Using this pattern of EIR, we observed that the predicted incidence of infection for the control cohort II was a little lower than that observed in the trial (Figure 4a). This discrepancy between the observed and predicted incidence was to be expected because of the higher transmission suspected in the Ilha Josina zone when compared with Manhiça.

In the control arm of cohort I, the model predicted an overall incidence of malaria fevers that was much higher than the number actually treated during the trial. This is because the simulation, based on a model fitted to intensive surveillance data from Senegal includes minor fevers that would not lead to treatment seeking and would elude detection in all but very active case detection. We assumed these fevers to be treated with a uniform probability and adjusted this probability until the incidence of treatment matched that of 0.5 episodes per child-year recorded in the trial. This was achieved by treating only 5% of the simulated fevers, and corresponded to an overall incidence of 4.2 episodes per child per year. This gave a similar time course of incidence of treated clinical episodes in the simulation to that observed in the trial (Figure 4b).

To simulate vaccination, we first considered which version of the model should be used for the action of the vaccine. All versions of our model predict that the effects of naturally acquired pre-erythrocytic immunity are negligible in pediatric populations, except in areas that are very highly endemic for malaria (Smith and others, Figure 1). The Mozambique trial consequently provides us with no information with which to decide between the two models A or B because the predictions for children are the same, whether the vaccine boosts naturally acquired immunity or acts independently of it.

Whatever value we choose to simulate for $X_v$ in model A, we can find a corresponding value for $\overline{VE}_0$ that would enable model B to match the predictions of model A in children. For instance, $X_v = 1,800$ inoculations in model A has almost exactly the same impact as $\overline{VE}_0$ in model B (Figure 1).

However, in older people with substantial exposure a vaccine operating by boosting the effect of natural exposure (model A) would have little or no effect (Figure 1) because the immune response is already saturated. This has the consequence that development of a malaria vaccine operating as described in model A would very likely be stopped before it was tested in children.

Model B implies that the underlying efficacy is age-independent. Since the RTS,S/AS02A vaccine has already shown efficacy in a group of 250 adult men in The Gambia, we argue that model A is a less plausible description of RTS,S/AS02A activity than is model B.

Using model B, a value of $\overline{VE}_0 = 0.52$ was needed to give $\overline{VE}_p = \overline{VE}_p = 0.45$ (Figure 4). When the vaccine arm of cohort II was simulated using this efficacy, the predicted incidence fell just below that observed in the trial vaccine arm. The fit was insensitive to the value of the parameter $b$ of the beta-distribution for $VE_0(i)$, providing this was not very low (Figure 5). A model with a very low value of $b$, corresponding to an all-or-nothing vaccine, cannot predict the observed incidence curves because it imposes an upper limit of $1 - \overline{VE}_0$ on the proportion of vaccinees who can become infected. In the trial cohort almost all the vaccinees eventually became infected. We consider it very unlikely that $VE_0(i)$ is constant and therefore adopted an intermediate value of $b = 10$ for all subsequent simulations.

**Figure 4.** Kaplan-Meier survival analysis of time to first events. **a**, Incidence of first patent infection: the step functions correspond to the results of the simulations, which did not consider the effects of infected children reporting to the health facility between the pre-set survey times. **b**, Incidence of recorded first clinical episodes (dotted lines indicate simulation). **c**, Incidence of first patent infection in a cohort of adults (simulation only).
Simulation and monitoring based on the Manhiça pattern of transmission and using the same health system and surveillance patterns as for the simulation of the Manhiça trial, adults 21–24 years of age.

The simulation of adults using the Manhiça pattern of the EIR as input, and the infection incidence curves (Figure 4c) were somewhat different shape to those observed in the Gambian trial, reflecting the different durations and intensity of the transmission seasons in the two sites. However, as in the Gambian data, the simulated curves for vaccine and control groups diverged initially, but seemed to indicate little or no efficacy in the second half of the surveillance period. As in the actual Gambian trial, a large proportion of both control and vaccine groups failed to show infections at the simulated cross-sectional surveys.

DISCUSSION

Simulation of the outcomes of malaria vaccine trials can help in the design of future trials by indicating which vaccine effects are likely to be measurable with relative ease and at what level of accuracy. It offers a means of deciding which outcomes are important, and also provides insights into vaccine action by indicating which results require further interpretation, and which can be explained by aspects of malaria biology included in the simulations. Such models can be used to predict population effects of a malaria vaccine, taking into account factors such as stage specificity, duration of effectiveness, effect of natural immunologic boosting, proportion of the population vaccinated, and prevailing entomologic conditions. In addition, models have a role in assessment and quantification of the impact of an efficacious malaria vaccine on public health and on the social and economic development of endemic areas.

An important issue for the design of pre-erythrocytic vaccines is whether delays in patency are of consequence even when the host eventually has a blood stage infection. Analyses of malarial therapy data indicate that the infecting dose has little or no impact on the severity of a P. falciparum infection, but in many infectious diseases the duration of latent periods are of importance for transmission dynamics.

RTS,S/AS02A must reduce the number of infected hepatocytes by a much higher percentage than its efficacy in blocking infections because even a single infected hepatocyte is sufficient to cause a blood stage infection. In RTS,S/AS02A vaccines given an artificial challenge, many infections that are not blocked completely take a few days longer to become patent than do infections in unvaccinated individuals, suggesting that they arise from a reduced number of infected hepatocytes. Our simulation of the effect of a five-day extension of the latent period in the vaccine group shows that such delays had a negligible impact on the results of the Mozambique field trial. It follows that an adequate simulation of this

Table 2

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<th>The Gambian trial</th>
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<tr>
<td>Prevention of infection</td>
<td>$\overline{V_E}_p$ 0.34 (0.08, 0.53)</td>
<td>$\overline{V_E}_p$ 0.33 (0.29, 0.37)</td>
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<tr>
<td>Prevention of clinical episodes</td>
<td>$\overline{V_E}_c$ 0.31 (−0.07, 0.56)</td>
<td>$\overline{V_E}_c$ 0.27 (−0.05, 0.49)</td>
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* Values in parentheses are 95% confidence intervals.
† Simulation and monitoring based on the Manhiça pattern of transmission and using the same health system and surveillance patterns as for the simulation of the Manhiça trial, adults 21–24 years of age.

Figure 5. Simulated Kaplan-Meier survival analysis of time to first infection for different distributions. The curves for vaccination correspond to different distributions for $V_E(i)$ (see Figure 2). In each case $\overline{V_E}_0 = 0.52$

Using the same model with $\overline{V_E}_0 = 0.52$ and $b = 10$, the simulation of cohort I gave an estimated efficacy of 33% against clinical episodes of malaria, which is very similar to the value recorded in the trial, 30% (Table 1). The simulated clinical episodes occurred rather uniformly in time during the surveillance period, again mimicking the data of the trial.

Our model was less successful in matching the trial results for severe malaria, predicting an admission rate for severe malaria of 33 per 1,000 child-years in the control arm, compared with the actual admission rate of 70 per 1,000 child-years. We therefore conjecture that almost all severe cases were identified, rather than only approximately half as initially assumed based on previous literature reviews. Such exceptionally high coverage, presumably resulting from increased awareness due to the trial, could also explain why mortality rates in the trial cohorts were very low.

As in the trial, predicted efficacy in preventing severe malaria was higher than that for prevention of any clinical episode, but was not as high as the actual value of $\overline{V_E}_0$ (although this estimate was imprecise) (Table 1).

We examined the reasons for these variations in efficacy in the simulations. In the model, the lower efficacy against clinical malaria episodes than against incidence of first P. falciparum infection was a consequence of a higher simulated pyrogenic threshold in the placebo group (geometric means of 1,617 parasites/μL compared with 1,243 parasites/μL in the vaccine group in a large simulation). Within our model, the pyrogenic threshold responds dynamically to $P. falciparum$, decreasing when individuals are not infected. This led to a small increase in the risk that an infection translated into a clinical attack in vaccinees whose exposure was reduced. The higher efficacy against severe malaria than against uncomplicated episodes was most obvious in the youngest children in whom there was the least naturally acquired immunity. We used the estimate of $\overline{V_E}_0 = 0.52$ to simulate a trial in a cohort of adults and compared the results to those of the Gambian trial in which protection could only be demonstrated for a short period (Figure 4c). This simulation gave very similar estimates of both $\overline{V_E}_p$ and $\overline{V_E}_c$ to those observed for RTS,S/AS02A among adult men in The Gambia (Table 2).

DISCUSSION

Simulation of the outcomes of malaria vaccine trials can help in the design of future trials by indicating which vaccine effects are likely to be measurable with relative ease and at what level of accuracy. It offers a means of deciding which outcomes are important, and also provides insights into vaccine action by indicating which results require further interpretation, and which can be explained by aspects of malaria biology included in the simulations. Such models can be used to predict population effects of a malaria vaccine, taking into account factors such as stage specificity, duration of effectiveness, effect of natural immunologic boosting, proportion of the population vaccinated, and prevailing entomologic conditions. In addition, models have a role in assessment and quantification of the impact of an efficacious malaria vaccine on public health and on the social and economic development of endemic areas.

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The close match of the model with the data therefore suggests that the interplay of vaccine-induced and naturally acquired immunity are the main causes of the variations in efficacy between the different outcomes and trials, and that neither selection biases nor random variation need be invoked to account for the differences. The simulation supports the use of our epidemiologic model to make predictions of the potential impact of introducing malaria vaccines into public health programs, but information about the duration of protection will be important in reducing the uncertainty in these predictions. Longer-term follow-up of trial participants is needed to evaluate the duration of protection. This information can be integrated into the models to simulate the public health and economic implications of malaria vaccine programs implemented together with other control strategies.

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