

Defining clinical malaria in vaccine trials

Because parasitaemia is often asymptomatic in endemic areas, not all sick individuals with parasites are really suffering from clinical malaria, but the greater the parasite density the more reasonable this assumption becomes. A case definition for clinical malaria for use in a trial can be obtained by defining a parasite density cutoff specific for the surveillance mechanism of choice (local health centre, hospital, active case detection). The sensitivity and specificity of different parasite density cutoffs is provided by modeling the excess risk of fever as a function of parasite density (Schellenberg *et al.* 1994; Smith *et al.* 1994b), and this has been used in a number of trials to decide upon an appropriate cutoff (Alonso *et al.* 2004; Alonzo *et al.* 1995; Genton *et al.* 2002)¹. Ideally this analysis is carried out in the same population (and age groups) as the vaccine trial and using the same morbidity surveillance system, since relationships between morbidity and infection depend on age and immune status (Mwangi *et al.* 2005; Smith *et al.* 1994a; Whitworth *et al.* 2000).

There are a number of outstanding issues in this approach. Consider the design of a trial in a population of children with an 80% prevalence of *P. falciparum* malaria, a distribution of parasite densities in the population (in the absence of vaccine or in a placebo group) as shown in the thick line Figure 1a and the relative risk of disease as a function of parasite density, as shown in the thick line in Figure 1b. Consider each of three hypothetical vaccine effects² (Table 1):

Vaccine A: (Pre-erythrocytic vaccine). This vaccine reduces the force of infection by 50%. For simplicity, we assume that this is reflected in a 50% reduction in the proportion of individuals who are infected across the whole range of parasite densities, and that a random sample of 50% of the clinical malaria episodes is averted³.

¹ The true efficacy is defined as $E = 1 - I_v / I_p$ where I_v is the case incidence in vaccinees and I_p is the case incidence in the placebo arm. The usual estimate of efficacy is $\tilde{E} = 1 - I_v(x) / I_p(x)$ where $I_v(x)$ is the incidence of cases at or above cutoff (x) in the vaccine arm, and $I_p(x)$ the corresponding incidence in the placebo arm. Assuming the specificity (ψ) of the diagnostic cutoff to be the same in both arms then an estimate of E adjusted for the effects of the imperfect case definition is:

$$\hat{E} = 1 - \frac{I_v(x) - (1 - \psi)(1 - \lambda)I_p(0)}{I_p(x) - (1 - \psi)(1 - \lambda)I_p(0)}$$

where λ is the attributable fraction in the placebo arm. A potential improvement in efficacy estimates is to thus to estimate \hat{E} from \tilde{E} , λ , and ψ and to use \hat{E} as an estimate of E . If ψ is sufficiently close to unity, then the differences between these two estimates is small. Exploratory analyses of the behaviour of \hat{E} suggest that it can be sensitive to x (Aponte, pers. comm), though it should not be so if the assumptions underlying its estimation are correct.

² The three hypothetical vaccines are not intended to correspond on a one-to-one basis to real vaccines, but rather to possible vaccine effects. A real (single-component) vaccine is likely to have secondary effects on the other measures in addition to its primary effect on either the force of infection, asexual parasite growth, or on elements of clinical immunity.

³ In a real trial the reduction in proportion of individuals infected varies over the trial period, depending on the time course of incidence, patterns of treatment with anti-malarial drugs, and on the variation between individuals in exposure to vectors and responses to vaccination. These factors significantly complicate the

Vaccine B: (Asexual blood stage vaccine). This vaccine reduces parasite densities by 50%, but does not affect the number of individuals who are infected, or the parasite densities at which they become ill.

Vaccine C: (Anti-disease vaccine). This vaccine is assumed to have no effect on parasite densities but to lead to an increase in the parasite density at which clinical malaria occurs.

Suppose that each of these vaccines is separately trialed using a standard Phase IIb design with (i) an equal number of placebo and vaccine recipients; (ii) a total of 100 parasitological slides in each arm used to estimate the effect on the parasite density distribution in the population, and (iii) a standardised clinical surveillance to detect fever cases, with a total of 100 cases expected in unvaccinated individuals.

As straightforward consequences of the primary effects of vaccination, the parasite density distribution in the community (Figure 1a) and/or the distribution in the cases (Figure 1b) differ in the vaccine arms of the trials from those in the placebo group (Table 2). This leads in turn to different relationships between incidence of disease and the community parasite density distribution, depending on the action of the vaccine (Figure 1c), and hence to different curves for the relationship between the attributable fraction, the frequencies of clinical cases with different densities, and the operating characteristics of case definitions (Figure 1defg, Table 2). For vaccines A and C the specificity of the case definitions is the same in the vaccine and placebo arms, while in the trials of vaccine B, the specificity is higher in the vaccine than the placebo arm.

For each of these models of vaccination random number generators were used to construct 500 simulated Phase IIb trials⁴ and thus to determine the distribution of efficacy estimates that would be obtained, and hence the power of the trials (the proportion giving statistically significant results). For this analysis, each of the category boundaries (x) used to sample the data was used in turn as a cutoff, and the average of the resulting efficacy estimates plotted against the cutoff (Figure 1h). At high values of x , corresponding to high specificity, the average efficacy estimate for vaccines A and C approaches the true efficacy, while for vaccine B (where specificity in the vaccine arm is higher than in the placebo arm) the efficacy is overestimated. The proportion of trials giving statistically significant results (Figure 1i) gives the power of the study. The power of the trials of vaccines A and C showed maxima at relatively low cutoffs, indicating that different cutoffs must be used if the aim is to optimise power, from that

analysis of relationships between infection and morbidity because, strictly speaking, the comparison should always be between contemporaneous data. This problem is particularly acute if parasites are cleared at the start of the trial, leading to complicated dynamics of infection and disease during the trial follow-up period.

⁴The simulated trial datasets were constructed by grouping the parasite densities into 19 categories. The community parasitaemia data were generated by defining multinomial probabilities for each of the 19 categories based on the data of Figure 1a and drawing a sample of 100 parasite densities from this distribution. The relative frequencies in Figure 1b were used to define the expected number of clinical cases for each of these 19 categories, on the assumption that the expected total number of clinical cases in the placebo arm was 100. The simulated number of cases in each category was then drawn from a Poisson distribution.

used to avoid bias in the estimate of efficacy⁵.

The difference in specificity of cutoffs between vaccine B and either placebo or vaccine A or C (Figure 1g) leads to the idea that perhaps different cutoffs should be used for vaccine and for placebo groups (Mwangi *et al.* 2005). To justify this in practice though, it would be necessary to demonstrate a statistically significant difference between trial arms in the specificity vs cutoff relationship. This would be a difficult statistical exercise, (because the specificity is estimated only indirectly), would lead to considerable difficulties in explaining the results especially if the efficacy proved highly sensitive to the choices of cutoffs in the different groups. Sample size is determined in order to give adequate power to measure the primary outcome (effect on case incidence) and thus most trials are too small to conclusively demonstrate whether the specificity vs cutoff relationship varies between arms.

Instead of defining cases on the basis of a single parasite density cutoff, an alternative is to estimate the total number of clinical malaria cases in each arm of the trial by assigning a probability to each fever case, rather than classifying each case dichotomously as above, or below, cutoff. The preferred estimation approach is to use a Bayesian latent class model to estimate the probabilities (Vounatsou *et al.* 1998)⁶, and it would be logical to carry out this analysis separately for both placebo and vaccine arms. The simulations of this approach presented in Table 2 suggest that it has comparable power to that of the cutoff method. This approach has not so far been used in analyses of clinical trials though it has been proposed as an alternative to the arbitrary choice of a cutoff (Rogers *et al.* 2006). It has been used in observational epidemiological studies (Smith *et al.* 1995; Vounatsou *et al.* 2000).

If such an approach were to be adopted as the primary analysis in a vaccine trial, it would be logical to carry out secondary analyses corresponding to the different panels in Figure 1. Such analyses would help to clarify whether the vaccine activity corresponds to that anticipated on the basis of the parasite stage that is targeted, and would highlight whether the primary measure of efficacy results from any unexpected behaviour in the parasitological and clinical data used to estimate it.

⁵Cutoffs for use in trials have generally been chosen to be high enough to ensure an adequate specificity to reduce bias in the estimated efficacy. From Figure 1i it is clear that this does not necessarily result in optimisation of study power. There is no reason why a threshold chosen to optimise study power should be particularly appropriate for any other purpose. In particular a diagnostic threshold optimised for use in a trial is not necessarily appropriate as a tool in clinical management (Rogers *et al.* 2006).

⁶The non-linear logistic regression model most widely used for defining the parasitaemia cutoff (Smith *et al.* 1994b) assumes a specific parametric form for the relationship between relative risk and parasite density. This can lead to severely biased estimates of the attributable fraction if the relationship happens not to conform to this pattern (Smith and Vounatsou 1997). This assumption is avoided in the latent class models proposed by (Smith and Vounatsou 1997) and (Vounatsou *et al.* 1998) which fit non-parametric curves for this relationship. Construction of interval estimates for the attributable fraction, the estimated malaria case incidence, or the trial efficacy is difficult (requiring bootstrap sampling) with the logistic regression approach, but relatively straightforward and robust within the Bayesian method (Vounatsou *et al.* 1998), for which software written in Winbugs (Spiegelhalter *et al.* 2003), is available from <http://www.sti.ch/en/research/biostatistics/downloads.html>

Table 1. Properties of hypothetical vaccines

		Vaccine effect			
		Placebo	A: Reduction in force of infection by 50%	B: Reduction in parasite densities by 50%	C: Multi- plication of pyrogenic threshold by 2
Prevalence of patent infection		80%	40%	77%	80%
Relative incidence of disease	All episodes	100.0	77.2	74.0	74.0
	Malaria	45.5	22.8	19.6	19.6
	Non malaria	54.5	54.5	54.5	54.5
True efficacy in preventing clinical malaria episodes		-	50%	57%	57%
True efficacy in preventing any disease episode		-	22.8%	26.0%	26.0%

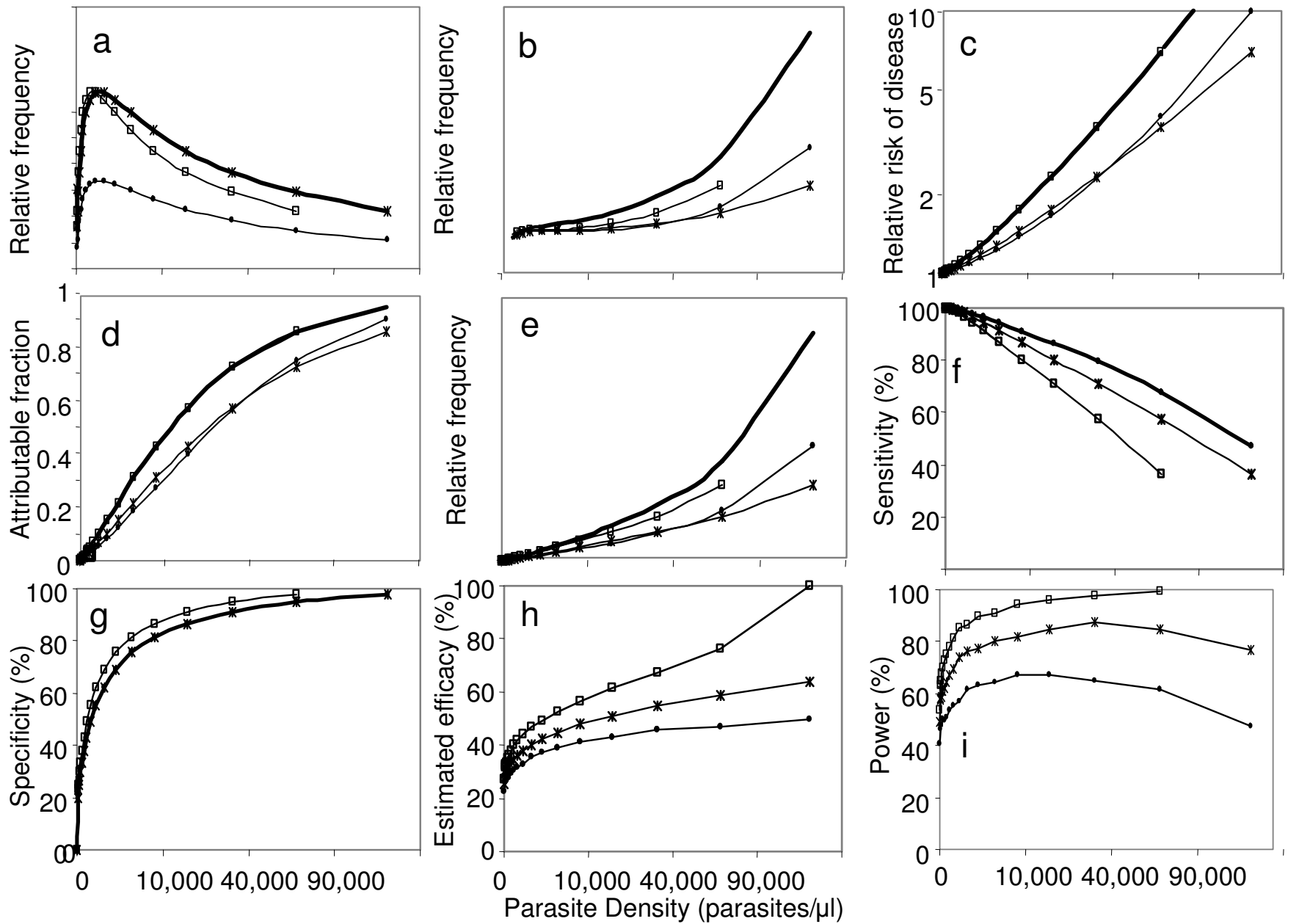
Table 2. Results of simulated vaccine trials

	Vaccine effect		
	A: Reduction in force of infection by 50%	B: Reduction in parasite densities by 50%	C: Multiplication of pyrogenic threshold by 2
Distribution of parasite densities in the community (Figure 1a)	Frequency is halved at each density above zero	Frequency of low parasite densities increases; frequency of high parasite densities decreases.	Same as placebo
Distribution of parasite densities in all disease cases (Figure 1b)	Frequency relative to that in placebo decreases with increasing parasite density	Frequency relative to that in placebo decreases with increasing parasite density	Frequency relative to that in placebo decreases with increasing parasite density
Relative risk of disease by parasite density (Figure 1c)	At any given density, reduced relative to placebo	Same as placebo	At any given density, reduced relative to placebo
Attributable fraction of cases by parasite density (Figure 1d)	At any given density, reduced relative to placebo	Same as placebo	At any given density, reduced relative to placebo
Distribution of parasite densities in clinical malaria cases (Figure 1e)	Frequency of high parasite densities lower than in placebo	Frequency of high parasite densities lower than in placebo	Frequency of high parasite densities lower than in placebo
Sensitivity of case definition, by parasite density (Figure 1f)	Same as placebo	At any given density, reduced relative to placebo	At any given density, reduced relative to placebo
Specificity of case definition by parasite density (Figure 1g)	Same as placebo	At any given density, increased relative to placebo	Same as placebo
Efficacy estimate* by parasite density cutoff (x) (Figure 1h)	Estimated efficacy increases with cutoff approximates the true efficacy at high cutoff values	Estimated efficacy increase with cutoff and exceeds the true efficacy at high cutoff values	Estimated efficacy increase with cutoff and approximates the true efficacy at high cutoff values
Power of study, by parasite density cutoff [#]	Reaches a maximum of about 67% at a cutoff of about 10,000/ μ l	Reaches a maximum of about 87% at a cutoff of about 40,000/ μ l	Increases to 100% at a parasite density of about 60,000/ μ l
Estimated efficacy using latent class model ^{&}	46.1% (18.7%)	55.6% (23.1%)	55.2% (16.5%)
Power using latent class model ($1-\beta$)	59.2%	82.4%	71.2%

*medians of a sample of $1-I_V(x)/I_P(x)$ in 500 simulated trials, where 500 simulated trials where $I_V(x)$ is the number of simulated cases in vaccinees, with parasite density $>x$; and $I_P(x)$ is the number of simulated cases in the placebo arm.

[#]based on a sample of 500 simulated trials, using binomial likelihood ratio tests (two sided) of the null hypothesis $I_V(x)=I_P(x)$ (significance level $\alpha=0.05$).

[&]Efficacy estimated as mean (standard deviation) of $1-I_V/I_P$ in 500 simulated trials where: I_V (case incidence in vaccinees) was estimated by fitting a latent class model to the data for the vaccine arm; I_P (case incidence in the placebo arm) was estimated by fitting a latent class model to the data for the placebo arm. The power is the percentage of trials in which the 95% credible interval for the efficacy shows no overlap with 0.



— Placebo ● A: Pre-erythrocytic vaccine ◻ B: Asexual blood stage vaccine * C: Anti-disease vaccine

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