The spread of antimalarial drug resistance: A mathematical model with practical implications for ACT drug policies

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Most malaria-endemic countries are implementing a change in antimalarial drug policy to artemisinin combination therapy (ACT). The impact of different drug choices and implementation strategies is uncertain. A comprehensive model was constructed incorporating important epidemiological and biological factors and used to illustrate the spread of resistance in low and high transmission settings. The model predicts robustly that in low transmission settings drug resistance spreads faster than in high transmission settings, and that in low transmission areas ACTs slows the spread of drug resistance to a partner drug, especially at high coverage rates. This effect decreases exponentially with increasing delay in deploying the ACT and decreasing rates of coverage. A major obstacle to achieving the benefits of high coverage is the current cost of the drugs. This argues strongly for a global subsidy to make ACTs generally available and affordable in endemic areas.

For the past half-century the malaria parasites of humans have been under tremendous selection pressure to evolve mechanisms of resistance to the prevailing antimalarial drugs. Chloroquine, and increasingly sulfadoxine-pyrimethamine (SP), have become largely ineffective as monotherapy for the treatment of *Plasmodium falciparum* malaria in much of the world. The World Health Organization (WHO) now recommends artemisinin-based combination therapy (ACT) as first line treatment for all *P. falciparum* malaria in endemic areas [1].

ACTs are efficacious, rapidly acting, well-tolerated and safe. They are available in various formulations which are generally given over three days. ACTs are effective against both asexual and early sexual parasite stages [2], and thereby reduce transmissibility [3,4]. The contribution of reduced transmissibility of individual treated infections to overall transmission depends on the proportion of transmissible infections that are treated and the degree of 'saturation' in the transmission dynamics. So far, significant *in-vivo* resistance to artemisinin derivatives has not yet been confirmed, and stable resistance has been very difficult to produce in the laboratory [5]. As for any combination therapy, which involves two effective drugs from different classes, both component drugs protect each other from the development of drug resistance whilst present at effective concentrations. This should prolong their useful lifespan provided that the individual components are not widely available as monotherapies [6].

Although malaria-endemic countries are switching to ACTs with increasing momentum, even at prices as low as US$1 per dose they are still too costly for communities and governments in poorer countries (5 – 10 times higher than the prices of chloroquine or SP in Africa [7]). Doubts have been raised about their actual operational effectiveness when they are implemented in "real-life" situations, where

infrastructures are weak, access to health care is poor, and there is widespread inappropriate use of antimalarial drugs [8]. Although providing easy access to very low cost ACTs in the private sector or free ACTs in the public sector may achieve this aim, it has to be balanced against the costs and risks of their widespread use. In particular, if artemisinins are used on their own and not in co-formulation with an effective partner drug, then there is a much greater risk of drug resistance arising to this precious class of drugs. Questions remain about the choice of combination therapy and timing of policy change. Finally, an important additional benefit of ACTs in low-transmission areas is their potential ability to reduce malaria transmission and thus the incidence of malaria. Enthusiasm for their deployment in high transmission settings is tempered by the expectation that their deployment is less likely to translate into reduced malaria incidence in these settings.

Modelling transmission dynamics and the spread of antimalarial drug resistance

The development of drug resistance is a two-step process, the *de novo* or the initial emergence of resistance and its subsequent spread. Resistance spreads because of the higher reproductive rate of resistance infections in the presence of antimalarial drugs. In this paper, we focus on modeling the spread of resistance assuming that drug resistance has already emerged among the human population. Combinations prevent resistance by preventing *de novo* emergence. Modeling the *de novo* emergence of drug resistance is discussed elsewhere [9,10].

Whilst there is a long history of modelling malaria epidemiology [11-13] (and the population genetics of drug resistance [10,14,15] [16]) none of the existing models incorporate all the important pharmacological, epidemiological, parasitological, and human behavioural factors that affect the effectiveness of drugs, the development of

drug resistance, and the transmission of malaria. More recent models have become more realistic although they were not developed primarily to study drug resistance [17-21]. We developed a complete model of the spread of antimalarial drug resistance, which incorporates all the important epidemiological factors, and we use it to evaluate different antimalarial policy options focusing on ACT deployment in low and high transmission settings.

This is a data-driven model; the model parameters for mosquito dynamics, malaria infection in the human host (asymptomatic, symptomatic and recrudescent infections) and immunity functions were obtained from clinical, laboratory and epidemiological studies and appropriate model fitting was then performed (Supplementary Information A).

At the outset, we assume a human population which has little or no exposure to malaria and therefore the population has no immunity to malaria. Infected mosquitoes bite randomly and infect humans with drug susceptible infections and a monotherapy is the only available treatment. As the population becomes exposed to malaria and gains some level of immunity, the model updates the age-stratified immunity of the population according to the Entomological Inoculation Rate (EIR) (which varies with vectorial capacity and the infective human population (see equation 8, Supplementary Information B)), and is allowed to run until a steady state is reached (Figure 1). At this point, resistance to the monotherapy is introduced and ACTs are deployed when resistance reaches a specified threshold. Resistance can then be tracked for a specified length of 10 years after steady state to gauge the impact on model outcomes over time. The model provides a number of outputs but we concentrate here on those relevant to policy. We present the proportion of infections

with resistant parasites, the malaria prevalence, and the incidence of malaria. Results of sensitivity analyses are presented in Supplementary Information C.

Results

Resistance, Transmission Intensity and ACT coverage

We explore model consistency and model sensitivity from the Coefficient of Variation (CV) and Partial Rank Correlation Coefficient (PRCC) respectively through four baseline scenarios at two levels of transmission intensity and two levels of ACT coverage i.e. low transmission setting with low ACT coverage (scenario A), low transmission setting with high ACT coverage (scenario B), high transmission setting with low ACT coverage (scenario C) and high transmission with high ACT coverage (scenario D) (Tables A9 and A10, Supplementary Information C). ACT coverage is defined as the proportion of ACT treatments among all symptomatic treated infections.

In the low transmission setting (EIR<1) with low ACT coverage variations in the estimated prevalence of malaria stay consistently high over time (CV ~ 90%) after the steady state is obtained, while the variation in the estimated resistance falls significantly by year 8 (CV ~ 9%) with resistance approaching fixation at 100% in our 10 year time horizon. In low transmission settings with high ACT coverage, the mean prevalence of malaria stays below 1% over 10 years. Migration plays an important role in sustaining malaria. Without imported cases, malaria is readily eradicated. Mean resistance increases at a slower rate than in a low coverage setting, reaching 80% in year 10. Compared to scenario A, variation in the estimated prevalence is slightly lower (CV ~ 75%) while variation in the rate of resistance is higher (CV > 30%). This indicates some levels of uncertainty in the consequences of deploying a

high coverage of ACT on the malaria prevalence and the rate of resistance. Once resistance to the slowly eliminated partner drug has emerged the spread of drug resistance and the malaria prevalence could be slowed down only by deploying ACTs at a high coverage rate while the resistance prevalence is still reasonably low.

In the low transmission setting, Vectorial Capacity (VC) was the most influential parameter affecting malaria prevalence (PRCC ~ 0.6), while VC (PRCC ~ 0.7) and the proportion of treated infections (PRCC ~ 0.4) were the most influential parameters affecting the spread of drug resistance.

In the high transmission setting (EIR > 100), malaria cannot be eradicated by antimalarial treatment of symptomatic cases alone because the major transmission reservoir is in asymptomatic persons who do not take antimalarial drugs. In the high transmission setting with low ACT coverage, malaria prevalence increases from 36% to 44% within 10 years due to the spread of resistance. Resistance spreads more slowly compared to the low transmission setting, reaching 80% in year 10. Variation in estimated prevalence is small (CV < 30%) while variation in the rate of resistance is consistently high (CV > 45%) compared to estimates in the low transmission setting. Both outcomes and their variations are unaltered by deploying a high coverage of ACT. Treatment of symptomatic infections in the high transmission setting has much less effect than in the low transmission setting. Consequently, the spread of drug resistance is driven by the fraction of the population with some residual antimalarial drug in their blood and not by treatment failure.

In the high transmission setting, the parameters influencing malaria prevalence are the characteristics of the infection in immune subjects with untreated infections. These are the parasite biomass (PRCC ~ 0.4), the gametocyte switch rate (PRCC ~ 0.4) and the duration of infection (PRCC ~ 0.3). The proportion of residual drug in the

population (PRCC ~ 0.9) is the dominant factor driving the spread of drug resistance in this setting. The strong correlation between the fraction of population with residual drug concentrations and the levels of resistance from this model suggests that controlling the use of presumptive treatment and encouraging the use of combination therapy with matching half-lives to reduce the selective window would slow the spread of resistance down within this setting.

Scenario A: Effects on resistance of delaying the policy change the ACTs

In the first scenario we consider the impact of varying the timing of switch to a high coverage (i.e. 85% of all symptomatic treated infections) by combining an artemisinin derivative with a failing partner drug (e.g. mefloquine) where the timing of the switch is governed by an observed prevalence of resistance to the partner drug in low and high transmission settings (Figure 2 and 3).

In the low transmission setting, treatment dependent parameter values are given in Table A8, Supplementary Information A. The force driving resistance comes from two sources; the first is from symptomatic malaria infections failing treatment and the second is exposure of infections to residual drug taken for presumptive or previous malaria treatment. More than 85% of all infections are symptomatic and thus treated. The rate of spread of resistance is faster relatively to the high transmission setting where treatment failure was identified to be the main force driving the spread of resistance. Deploying a high coverage of effective treatment, such as an ACT, when the level of resistance is still low delays the spread of drug resistance, a result consistent with previous results from simple epidemiological models [22].

In the high transmission settings, immunity prevents most acquired infections transmitting (and synergises with antimalarial drugs). Approximately 94% of all

infections are asymptomatic and untreated. In the absence of antimalarial treatment, the resistant infections have no survival advantage over the sensitive ones, and may have a fitness disadvantage. The main driving force for resistance is created from the selective filter provided by people carrying low residual concentrations of drugs, which protect against the establishment of new sensitive infections but not the resistant ones [23,24]. Without this residual drug effect, the rate of resistance would be much lower than shown in Figure 2b. Residual drug levels come mainly from previous 'presumptive' treatments (normally for other febrile illness), and are largely unrelated to the peaks of parasitemia [9] and are thereby assumed to have little or no influence on the *de novo* resistance selection probability.

Combining an artemisinin with a drug to which resistance has arisen delays the spread in resistance in the low transmission setting (Figure 2a and 3a) but this delay decreases exponentially the later the switch is made to the ACT. By contrast, in the high transmission setting (Figure 2b and 3b), varying the time to switch to the ACT has only a small impact on delaying the spread of resistance.

Scenario B: Effects on artemisinin resistance of different levels of ACT coverage

In this scenario we assume that the monotherapy is the artemisinin and that drug resistance emerges to the artemisinin compound, rather than to the partner drug in an ACT (e.g. either piperaquine or lumefantrine), which is assumed to remain effective (see Table A8, Supplementary Information A). This simulates the current scenario in places such as Cambodia where artesunate monotherapy use is widespread [25]. We assume that the switch to the combination therapy is made when the resistance to artemisinin is as low as 1% (Figure 4). If the switch is made very early, when there are still very few cases of drug resistance, then the higher the coverage with the ACT,

the greater is the delay in the spread of resistance. At coverage rates of >80%, the level of resistance to the artemisinin does not reach 50% within the time span of 10 years. In general, the impact of ACT deployment on malaria incidence and prevalence is as expected. By deploying ACTs at a high coverage, the prevalence of malaria can be kept at a very low level over time (0.5%) and incidence is less than 50 cases per year, indeed in the model eradication is only prevented by the influx of malaria in immigrants (Figure 5). Similar to the first scenario, the impact of ACT in the high transmission setting is much less.

The model shows that deploying ACT in a high transmission setting has a small impact on the spread of resistance and malaria prevalence. However, as one of the model outcomes (results not shown), treatment failure in both low and high transmission settings can be sustained to a low level by deploying high ACT coverage (25% of recrudescence compared with 10% when deploying high ACT coverage at year 10). In order to make an impact on malaria transmission and resistance, vector control strategies need to be applied to reduce the vectorial capacity. Reduction in the transmission intensity results in fewer infections which, as host immunity declines, are more likely to be symptomatic and eventually this makes malaria control by drugs more effective.

Discussion

A mathematical model always represents a simplified version of the true biological system. Complexity is traded against robustness. We have developed a model of suitable complexity to include all important features of malaria transmission and the spread of antimalarial drug resistance in *P.falciparum*. The model predicts rapid spread of drug resistance in low transmission settings, and slower spread in high

transmission settings. This is consistent with epidemiological observations. In low transmission settings a higher proportion of potentially transmissible infections are exposed to antimalarial drugs and lower immunity increases the individual probability of treatment failure and transmission of resistant parasites. In low transmission settings, the spread of drug resistance can be slowed by combination treatments in which two or more effective drugs, which do not share resistance mechanisms, are combined. ACTs are currently the combinations of choice. This switch to combination treatment needs to be made early in the evolution of drug resistance with high rates of coverage (> 80%) if the full benefits in terms of delaying resistance are to be realised. This is the case whether the monotherapy to which resistance arises is an artemisinin derivative, or a non-artemisinin drug. The structure of this model allows many other policy relevant questions related to malaria control (vector controls, drug adherence and intermittent presumptive treatments etc) to be addressed. However, as a population based model, the ability to assess the effects of individual variation and the incorporation of important pharmacokinetic and pharmacodynamic variables is limited.

In low transmission settings increasing ACT coverage is essential if the dramatic effects on malaria incidence observed recently in Northwest Thailand, KwaZulu Natal and Zanzibar are to be extended to other areas [26,27]. *P. falciparum* malaria can be eliminated, although microheterogeneities in transmission intensity in remote areas will often ensure a protracted “end–game”. The impact of ACTs on drug resistance in high transmission intensity settings is limited because the majority of population are immune, many infections are asymptomatic, and therefore a smaller proportion of the infections are treated. An important weakness in our understanding of the epidemiology of malaria is the relative contribution of asymptomatic and

symptomatic infections to transmission. The smaller the contribution of asymptomatic infections, the greater is the effect of ACTs in slowing resistance spread. The main force driving the spread of drug resistance in these circumstances is the chemoprophylactic effect of presumptive therapy, which provides a selective filter for resistant parasites. The model also predicts that at low transmission intensities malaria transmission is readily eradicated without the continued influx of infected migrants. This has important implications for eliminating malaria [28,29].

The question of whether it is possible to reduce malaria transmission sufficiently to eliminate malaria eventually in high malaria transmission areas remains unresolved [8]. In high transmission settings, this model predicts that high ACT coverage alone cannot reduce malaria transmission unless it is used together with vector-control measures i.e. use of insecticides and deployment of insecticide-treated bednets (ITN) and other materials to reduce the force of infection. To overcome the obstacles to high coverage of the unaffordability and unavailability of ACTs, it has been argued persuasively that provision of global subsidies for co-formulated ACTs must be provided at the top of the distribution chain. This would facilitate considerably the flow of drugs down to the end users through the existing public and private sector distribution pathways, with the ultimate objective of making effective antimalarial treatments available and affordable even to the poorest people [30]. Stabilizing demand for ACT would also create incentives for ACT production, resulting in lower prices [7].

Methods

Model development and parameter estimation

We model transmission and antimalarial drug resistance using a dynamic, age-structured population-based model where the introduction of new imported (migrant) infections varies stochastically each day. At the end of each iteration, the inoculation rate for the human population is calculated from the infective contact with vectors and host susceptibility, based on the formula given by Dietz [31] (see equation 1, Supplementary Information B). The human infectiousness of infected Anopheline mosquito feeds is estimated from the non-linear relationship between gametocyte density and the chance of infecting mosquitoes, formula given by Jeffery and Eyles [32] (see equation 5, Supplementary Information B). The average gametocytemia depends on the average parasite biomass by age group and the estimated gametocyte switch rate (i.e. probability that an asexual parasite develops to a sexual parasite). The size and age-structure of the human population was assumed to be constant over time in the model and was based on an average African age-structure (http://esa.un.org/unpp).

The model handles malaria like a "macro" parasite by quantifying the density of infection in the human host. The *in-vivo* effect of drugs on parasite density can be measured, allowing quantification of the pharmacodynamic properties of antimalarial drugs [33]. Different multiple linear and non-linear regressions equations as a function of age and EIR were fitted to data from age-stratified epidemiological studies in areas with different transmission intensities. Stepwise selection using the Akaike Information Criteria (AIC) was used to identify the best fit in the case of non-nested functions and the maximum-likelihood ratio test was used for nested functions. These relationships represent the development of immunity with age and malaria exposures. Different facets of malaria immunity are incorporated into this model (Figure 6) i.e. reducing host susceptibility to infection [34,35], reducing the level of (largely asymptomatic) parasitemia in infected people [36,37], reducing the likelihood of fever

and other symptoms in infected patients [38-40], reducing the treatment failure rate for a particular level of antimalarial drug resistance [41-46], and increasing the recovery rate of an established infection i.e. shortening the duration of infection [47-49] (Table A7, Supplementary Information A). Some immunity functions were not measured directly from epidemiological studies (i.e. host susceptibility, duration of infections and treatment failure), so a normalized function of age-stratified parasite density was used to estimate the relationship between age and host susceptibility and between age and duration of infections by specifying initially the "maximum host susceptibility" in a non-immune person and the "maximum duration" of *treated* and *untreated* infections. The host susceptibility and the duration of infection for any given age group in any transmission intensity setting are then determined by the shape of the immunity curve. As the shape of the age effect on treatment failure is similar to the relationship between age and severe malaria [41], we apply the same technique to the normalized function of age-stratified risk of severe malaria to adjust the treatment failure rates for any given age group in any transmission intensity setting. The maximum value of duration of infection and the maximum value of treatment failure are dependent on treatment type, drug resistance, and patient adherence to therapy.

For simplicity, the gametocyte switching rate (GSR) was assumed to be uniform among asexual parasites and infections but to vary depending on the type of drug, and also between primary and recrudescent infections. It is assumed that all humans are equally attractive to biting mosquitoes and that all mosquito biting vectors are equally susceptible i.e. the infectiousness to mosquitoes is assumed to be determined solely by the gametocyte density in humans.

To track the rate of spread of resistance, the inoculation rates of resistant and sensitive infections were calculated separately. Thus, the proportion of infected and

infectious sensitive infections is the sum of all drug - sensitive infections treated with different treatments multiplied by their mean infectiousness. Similarly, the proportion of infected and infectious drug - resistant infections is the sum of all resistant infections treated with different treatments multiplied by their mean infectiousness. Both were then divided by total number of infections (see equation 2 – 3, Supplementary Information B). The population with selective residual antimalarial drug concentrations focused on this model is the proportion with concentrations in the blood which prevent establishment of new drug sensitive infections but allow establishment of resistant infections. This proportion of population was based on published literature (equation 6 – 7, Supplementary Information B).

Model simulations and outputs

Malaria infections initially are all drug-sensitive and symptomatic humans receive only monotherapy (drug A). A steady state is defined as the point at which the number of new malaria cases (i.e. excluding imported cases) has varied day to day by less than 1% over a year. When steady state point is reached, the resistance to drug A is introduced, either as importation of a small number of resistant infections or by the *de novo* emergence of resistance based on available clinical and laboratory data. Artemisinin or its derivatives, or a completely new drug can also be introduced and used in a combination with drug A, or it can be used as a monotherapy. Any changes in malaria prevalence and levels of drug resistance thereafter are assumed to result from the impact of the different treatment strategies. The relationship between drug resistance and the maximum failure rates in a non-immune host was defined depending on the infection, type of treatment, and likelihood of adherence. Adherence is incorporated in the model by adjusting down the expected failure rates of treated

infections. Multiple recrudescences are treated as one continuous recrudescence, and overall infectiousness is calculated from the area under the gametocyte – time curve (AUCgam). For each model iteration, the outcomes in terms of estimated EIR, proportion of symptomatic infections, proportion of treatment failure, malaria prevalence and percentage of resistant infections are estimated for a period of 10 years from the steady state.

The sensitivity of the model was tested in the four baseline scenarios (scenario A – D). The details of fixed and variable parameters with their respective distributions are given in Tables A1 – A6, Supplementary information A. Each scenario is repeatedly run for 5,000 simulations with a unique set of parameters selected using the Latin Hypercube Sampling technique (LHS). The Coefficients of Variation (CVs) which determine the uncertainties of the model outcomes (Table A9, Supplementary Information C) and the Partial Rank Correlation Coefficients (PRCCs), which identify influential factors, were calculated (Table A10, Supplementary Information C). Full technical details can be found in the author's thesis [50].

Note: Supplementary information is available on the website.

Abbreviations

SP, sulfadoxine - pyrimethamine;
ACT, artemisinin combination therapy;
GSR, gametocyte switching rate, proportion of asexual parasites committing to sexual stage differentiation;
EIR, entomological inoculation rate;
VC, vectorial capacity;
AIC, the Akaike information criterion;
AUCgam, area under the curve of time versus blood gametocyte density;
PRR, parasite reduction ratio, fractional reduction in parasitemia per asexual cycle;
LHS, Latin hypercube sampling;
CV, coefficient of variation;

PRCC, partial rank correlation coefficient;
ITN, insecticide-treated bednet

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Competing Interests

NJW is chairman of the WHO antimalarial treatment guidelines committee.

Author Contributions

WP developed and programmed the model and co-wrote the paper.
SY developed the model, focusing on parameterization and co-wrote the paper.
IH provided intellectual guidance on the mathematical modelling.
AD provided suggestions on parasite dynamics and edited the paper.
ND provided input on model design.
NJW conceived of the study, provided overall intellectual guidance and edited the paper.

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Figure legends

Figure 1. Schematic diagram of the biological model progression from steady state through to the introduction of resistance and changes in drug policy

Figure 2. The predicted spread of resistance to mefloquine when combined with artesunate at different level of resistance. Figure 2a shows the result in low transmission setting and Figure 2b shows the result in high transmission setting, assuming 85% coverage of the ACT (mefloquine and artesunate). The dotted line shows the 50% resistance. Each curve represents the mean of ten simulations.

Figure 3. Delay in the spread of resistance to mefloquine by combining it with artesunate. The delay in the development of resistance is measured as the proportional increase in time to 50% resistance compared to the continued use of mefloquine as a monotherapy.

Figure 4. The spread of artemisinin resistance at different levels of ACT coverage. Figure 4a shows the spread of artemisinin resistance at varying levels of ACT coverage from 0% (i.e. use of artemisinin monotherapy) to 100% use of ACTs. Each line represents the mean of ten simulations. The dotted line shows the 50% resistance level. Figure 4b shows the delay in resistance measured as the increase in the time to 50% resistance (t_{50}) compared with the t_{50} when using artemisinin monotherapy. The dotted line represents an extrapolation of the curve when the resistance does not reach 50% within the 12-year timeframe.

Figure 5. Change in malaria prevalence and incidence at different rates of coverage with ACT

Figure 6. Schematic diagram of how immunity influences age-stratified likelihoods in the biological model.

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FIGURE 1: Schematic diagram of the biological model progression from steady state through to the introduction of resistance and changes in drug policy

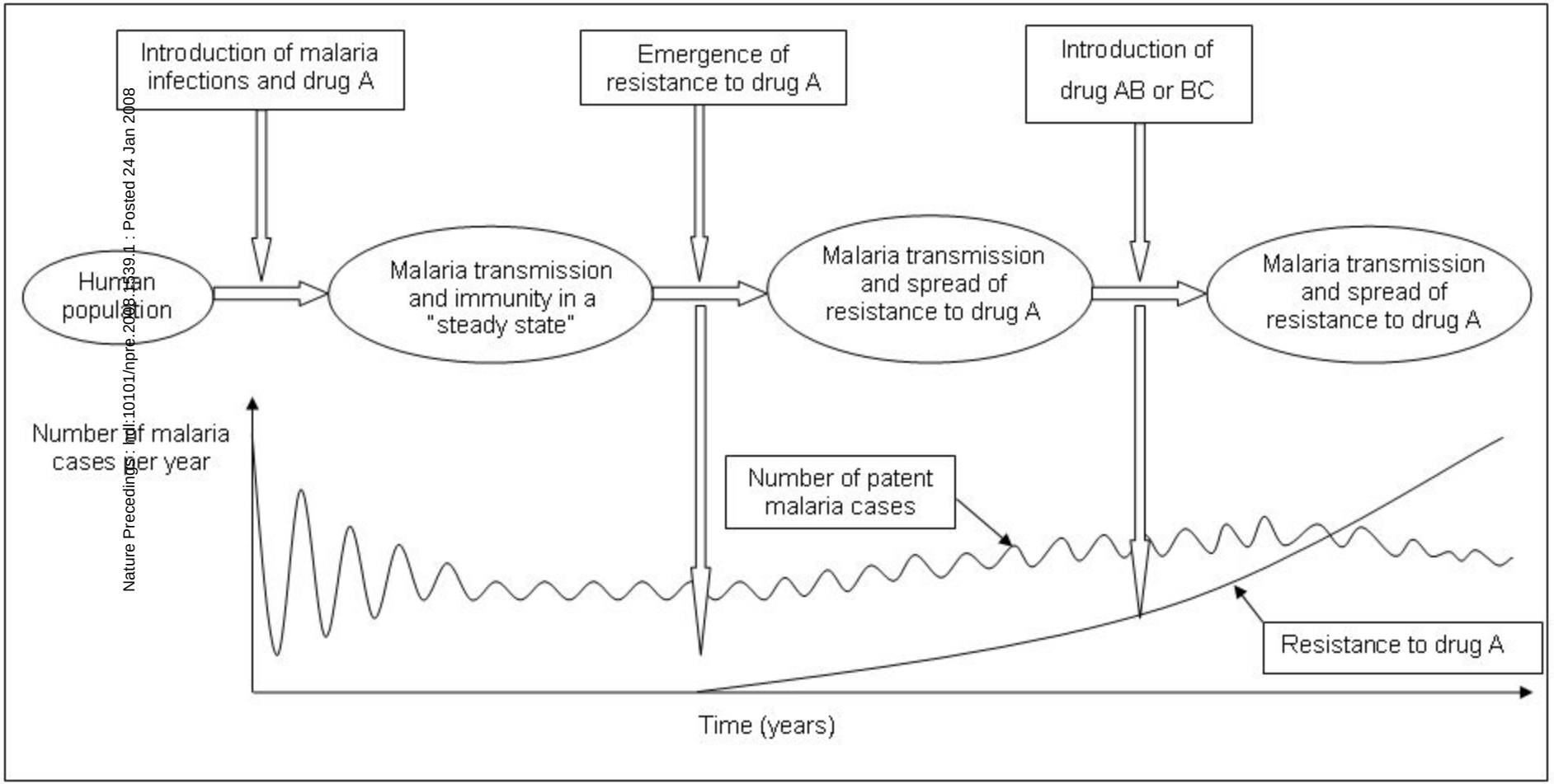

FIGURE 2: The predicted spread of resistance to mefloquine when combined with artesunate at different level of resistance. Figure 2a shows the result in low transmission setting and Figure 2b shows the result in high transmission setting, assuming 85% coverage of the ACT (mefloquine and artesunate). The dotted line shows the 50% resistance. Each curve represents the mean of ten simulations.

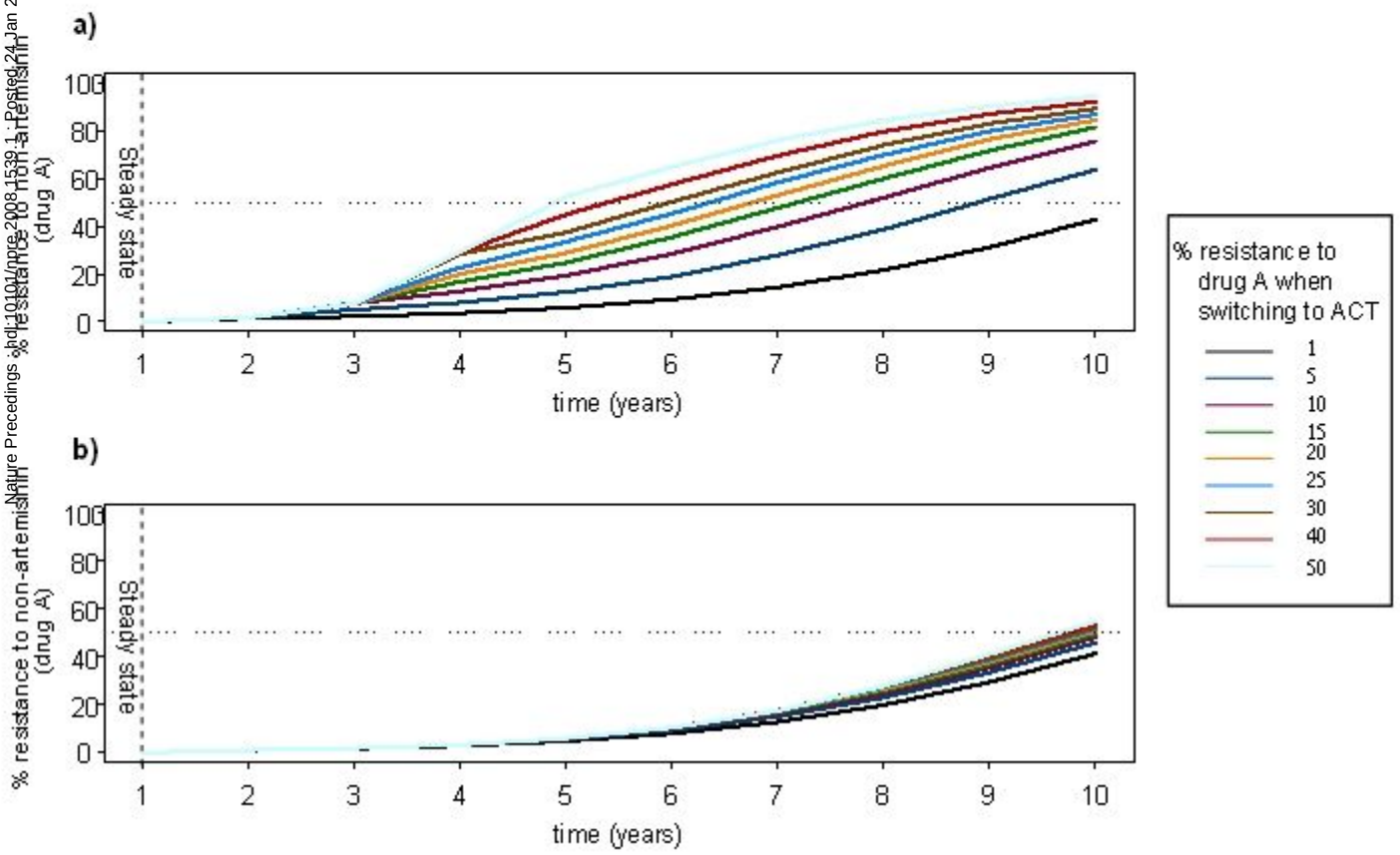

FIGURE 3: Delay in the spread of resistance to mefloquine by combining it with artesunate. The delay in the development of resistance is measured as the proportional increase in time to 50% resistance compared to the continued use of mefloquine as a monotherapy.

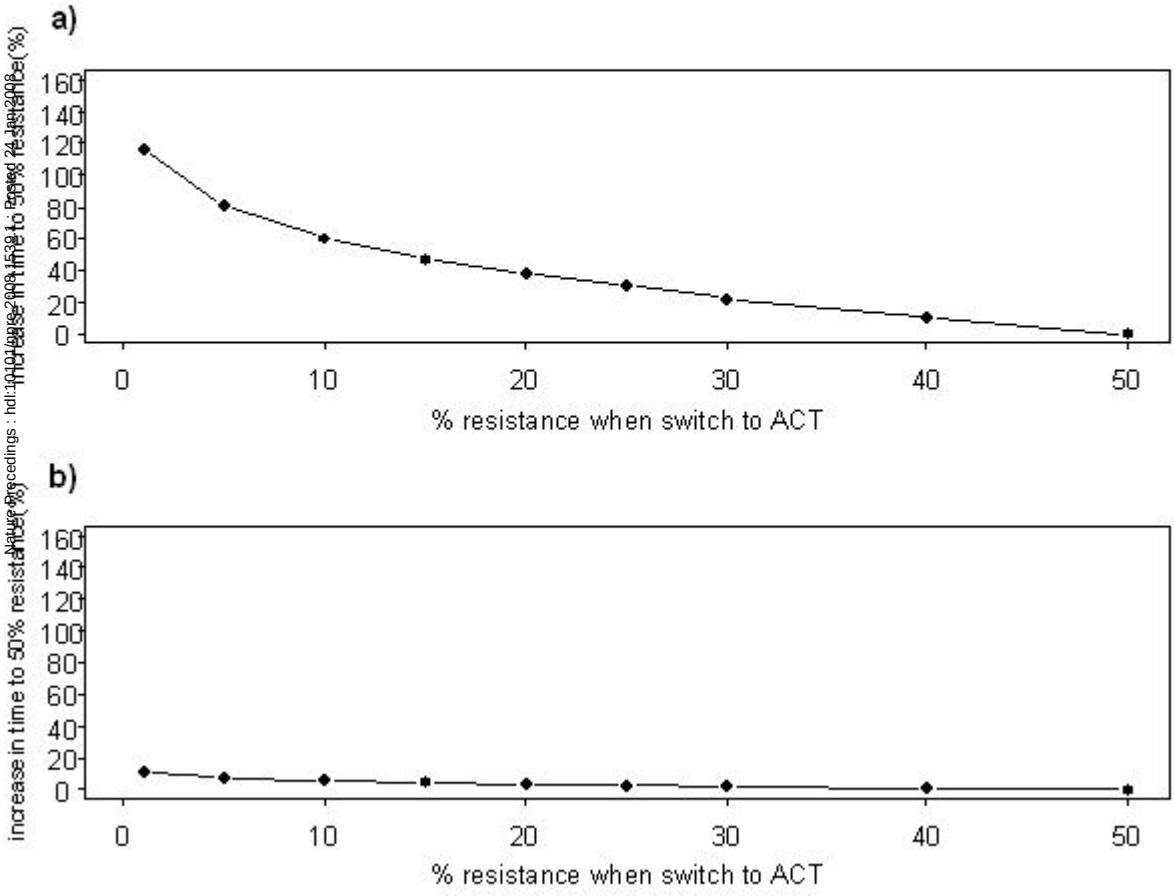

FIGURE 4: The spread of artemisinin resistance at different levels of ACT coverage. Figure 4a shows the spread of artemisinin resistance at varying levels of ACT coverage from 0% (i.e. use of artemisinin monotherapy) to 100% use of ACTs. Each line represents the mean of ten simulations. The dotted line shows the 50% resistance level. Figure 4b shows the delay in resistance measured as the increase in the time to 50% resistance (t_{50}) compared with the t_{50} when using artemisinin monotherapy. The dotted line represents an extrapolation of the curve when the resistance does not reach 50% within the 12-year timeframe.

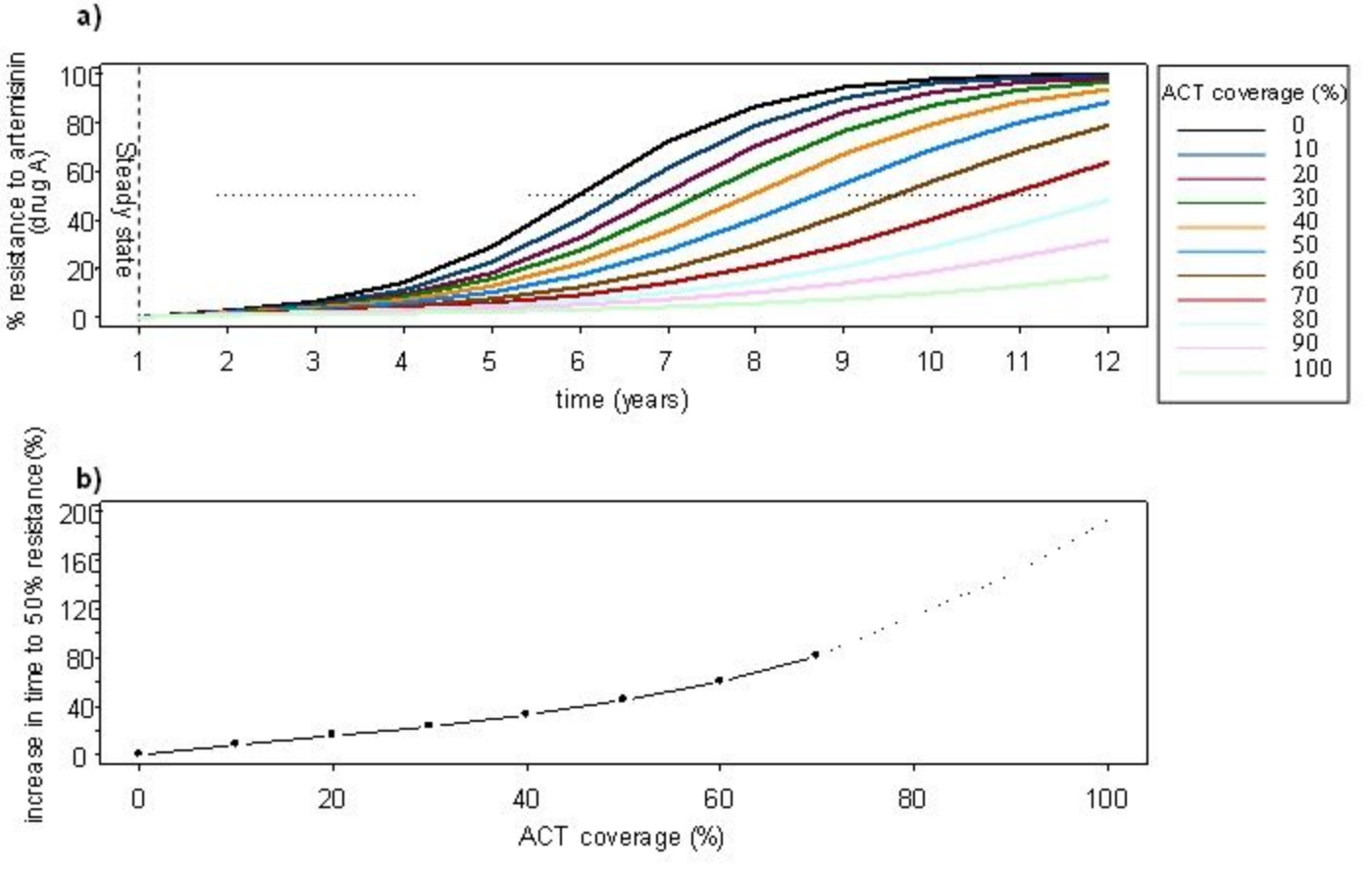

FIGURE 5: Change in malaria prevalence and incidence at different rates of coverage with ACT

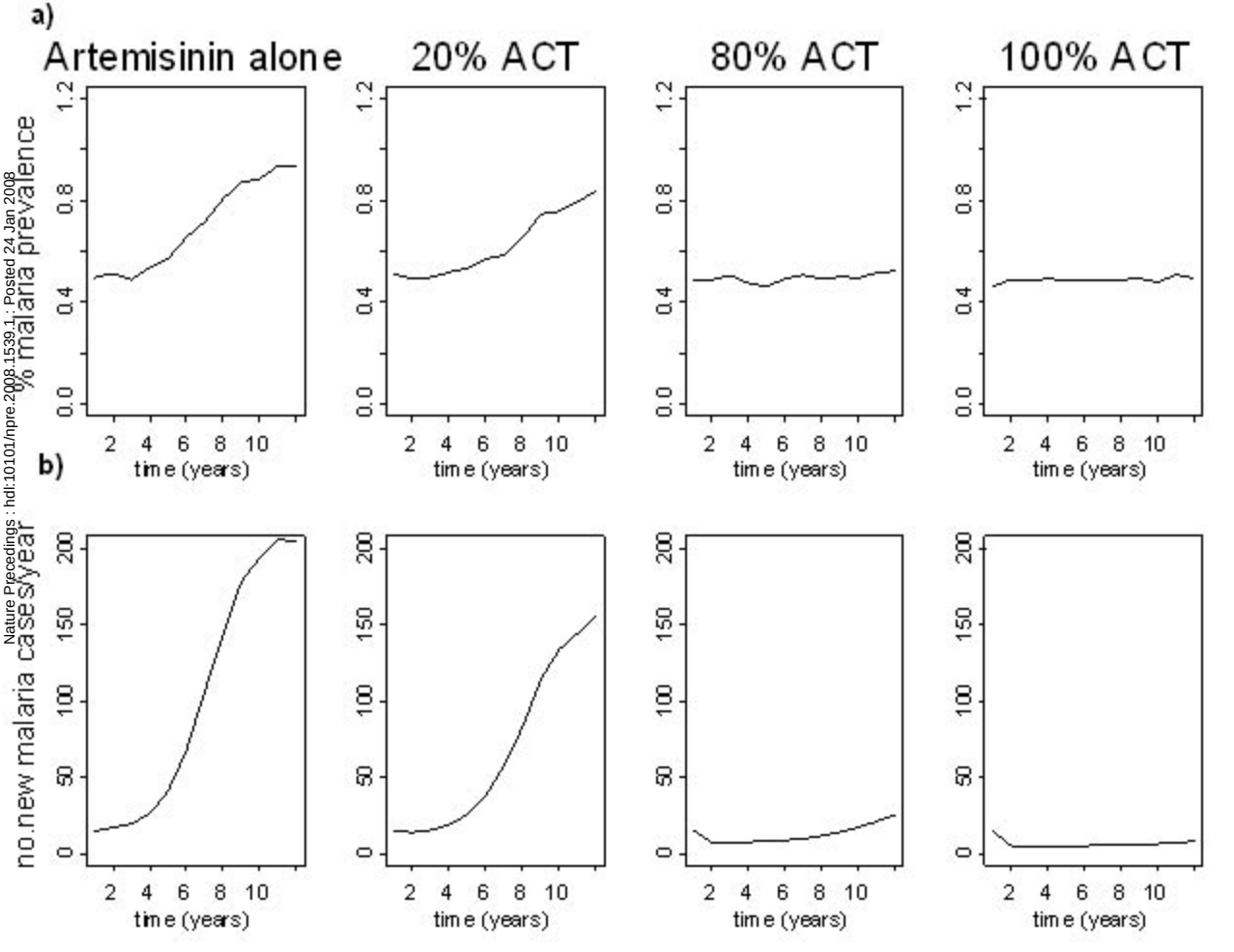

FIGURE 6: Schematic diagram of how immunity influences age-stratified likelihoods in the biological model.