Effects of some Biological Covariates on the Probability of First Recurrence of Malaria following Treatment with Artemisinin Combination Therapy

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Abstract: Many investigations have shown that artemisinin-based combination therapies are effective in the treatment of uncomplicated malaria and that they do not increase parasite resistance to treatment as much as treatment with single substance. We study the relation between some biological covariates and the time to first recurrence of malaria for children treated for malaria in a clinical trial. One group received artesunate plus sulfadoxine-pyrimethamine and the other only sulfadoxine-pyrimethamine. We consider the event malaria-free for the first 42 (and 84) days. We use logistic regression models for the analyses. The main results show that the probability of no recurrence is higher if the parasite density in the blood is high. The results are inconclusive for other explanatory biological variables. The infecting parasites having genes that indicate resistance, gave different results at the two different treatment centres. There was no appreciable difference in the effects of treatment over the two follow-up periods and these treatments do not have any effect on the probability of a recurrence.

Keywords: Logistic model, clinical covariates, malaria treatment, parasite density, drug resistance, genotype, incomplete data.

1. INTRODUCTION

Artemisinin-based combination therapy (ACT) is strongly recommended by WHO for the treatment of uncomplicated *P. falciparum* malaria. The fast acting artemisinin-based compounds are combined with a partner drug to delay or prevent emergence of resistance. The artemisinin derivative in the combination kills parasites faster but has a short half-life, while a partner drug in this combination which has a longer half-life, clears the remaining parasites after the artemisinin is no longer present [1-3].

In our previous work on efficacy of ACTs [4], we arrived at the conclusion that artesunate plus sulfadoxine-pyrimethamine (ASP) was a better treatment for children below 5 years of age than only sulfadoxine-pyrimethamine (SP). This confirms the reason behind the advocacy of artemisinin-based combination therapy for the treatment of malaria. We showed that the probability of treatment success (no recurrence within 42 days) was relatively high. In case of treatment failure, recipients of ASP stayed free from malaria for 6.2 days longer on the average (with 95 % confidence interval of 4.2 – 8.3 days). To see if there were any effects remaining after 42 days, we also compared the outcome of a follow--up period of 84 days. There were more early recurrences when children were treated with SP alone. There were still some recurrences after 42 days and there were reasonable probability values of recurrence. The estimated malaria-free period those with a recurrence was 5.4 days longer on the average (with 95 % confidence interval of 1.6 – 9.2 days) for ASP. The data came from a clinical trial conducted in Tanzania in 2004 to compare the efficacy of ACTs with alternative treatments without artemisinin. For a better understanding of this paper, we will give a brief description of the study in Section 2 but a detailed description is found in [4, 5].

In the article [5], we developed a probability model for the estimation of haplotype frequencies and used it to compare the proportion of parasites with resistance genes at baseline and at first recurrences, and also

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study the effects of malaria treatment on parasite drug resistance. Results in that article confirmed that ACTs are more effective in treating malaria without increasing drug resistance in the remaining parasites. Treatment with only sulfadoxine-pyrimethamine did not clear all the parasites with resistant genes and surviving parasites were responsible for the reappearance of malaria. We showed that the proportion of parasites with resistant genes increased between the original infections and the recurrences for two of the three studied sites. In those sites the relative proportion of resistant genes doubled. The results were significant but the intervals were quite wide. Treatment with sulfadoxine-pyrimethamine in combination with artesunate was effective and all observed first recurrences depended on new infections.

Mårtensson, [1, 6], used the data of the trial to assess the influence of consecutive-day blood sampling on polymerase chain reaction (PCR)-adjusted parasitogical cure rates after stepwise genotyping of merozoite surface proteins 2 *(msp2)* and (*msp1*). This was then compared with a standard protocol using paired blood samples only. Their argument was that analyses on blood samples collected at the day of enrollment and on days of recurrent parasitaemia, do not reveal the daily dynamics of *Plasmodium falciparum* in endemic areas.

In this paper, we study the effects of some biological covariates together with a time covariate, in relation to first recurrence or non-recurrence of malaria. We study how some covariates, for instance, drug type, number of resistant and sensitive genes, mixed infections, time, number of parasites in each patient's blood sample prior to start of treatment and age will affect the probability of a first recurrence of parasites. Earlier results found in [4], showed that there was a difference in effect of treatment over the periods (0 – 42) and (0 – 84) days. Our focus now is on the effects of covariates on the chances of a recurrence of malaria in children taking treatment (ASP or SP) for some follow--up periods: (0 – 42), (42 – 84) and (0 – 84) days, respectively. Is there a difference over these periods, when we consider ASP/SP with other biological covariates? In investigating if there is a difference in effect of ASP and SP during the periods (0 – 42) days and (42 – 84) days, we exclude information on all children who have had a recurrence before day 42.

The models we want should be complex enough to fit data well but also simple to interpret. Even if the models do not fit data well, they should contain important biological covariates, whose effect on the probability of getting cured are of interest. It is unrealistic to hope that we can find a true model for any data set.

2. BRIEF DESCRIPTION OF THE CLINICAL STUDY AND METHODOLOGY

2.1. Participants

The clinical trial was conducted in 2004 at two health centres in Tanzania: Uzini and Konde with 206 and 178 uncomplicated malaria patients, respectively. The trial was supervised by the Karolinska Institutet (Sweden).

Admission criteria into the study included a blood sample positive for *Plasmodium falciparum* at a density of between (50 - 5000/200 WBC) or (2000 - 200000) asexual parasites/mL of blood, body weight ≥ 6kg, age <5 years, axillary temperature of at least 37.5^{o} or a history of fever during the past 24 hours, access to study site, and be able to show up during follow-up [6].

2.2. Treatment Regimens and Follow-up

The parasitaemia children recruited for the study were randomly allocated into two treatment arms. In one arm, the children were treated with only sulfadoxine-pyrimethamine (SP) and in the other arm they were treated with artesunate plus sulfadoxine-pyrimethamine (ASP). Sulphodoxine-pyrimethamine (Fansider) is a co-formulated drug, each tablet containing 500 mg sulphodoxine and 25 mg pyrimethamine. Though, it consists of two different compounds, it is not considered a combination therapy.

The sick children were tested for parasites on Day 0, using microscopy on stained thick-blood films and quantified in terms of parasites per millilitre. Each outpatient guardian was asked to bring the patient to the study site on days 7, 21, 28, 42, 56 and 84 for clinical and laboratory assessments.

If a child had a fever, all tests and examinations were ran as on Day 0. In that case, the same procedure was made as for ordinary follow-ups. Children could be withdrawn or excluded from the study for a variety of reasons. Among the exclusion criteria were, showing signs and symptoms compatible with severe malaria, intake of any other anti-malarial drug outside the protocol, serious adverse effect, and

haemoglobin level < 50g/L. In the case of severe malaria, the patient was given rescue treatment and withdrawn from the study.

2.3. Parasite Genotyping

Polymerase chain reaction (PCR) genotyping of merozoite surface protein 2 *(msp2),* considered the most informative single genetic marker for multiplicity of *Plasmodium falciparum* infections, was performed to differentiate reinfections from true recrudescence. The parasites in the blood samples were analysed and the single-nucleotide polymorphisms (SNPs) at three positions in the *pdhfr* gene were determined. The three positions (*pdhfr* 51, 59 and 108) could be defined as either resistant (R) or sensitive (S). These positions were known to be important for the resistance of the parasite to sulfadoxine-pyrimethamine. If both parasites with R and S SNPs were present, this was denoted by the letter M. Each blood sample was classified for its parasites *pdhfr* characteristics by three letters from SSS to MMM, denoting the status of each one of the individual SNPs. For example, RSM means that the child had only parasites with resistant SNPs at the first position and only sensitive SNPs at the second position, but there were both parasites with resistant and sensitive SNPs at the third position.

The gene sequence at baseline and at the first recurrence of malaria was determined, which could have been a reinfection or a recrudescence. In order to circumvent the ambivalence in distinguishing between reinfection from recrudescence, we consider all first recurrence of malaria within the study period as a first recurrence of malaria. Though some of the children suffered a resurgence of the disease more than once during the follow-up period, in the analysis we focus only on the first time malaria parasites are detected in their blood samples. That is, we record each genotype at baseline and the corresponding genotype (if any) at first recurrence of the disease and take note of the changes in positions of the genes.

3. FORMULATION OF STATISTICAL MODEL

In this section, we formulate the model that will be used throughout this article. Logistic regression technique is increasingly used in medical and epidemiological research. It extends the techniques of multiple regression analysis to study situations where the outcome variable is categorical, that is, take on one of two possible values. The usual regression model is not appropriate when the dependent variable Y is a dichotomous variable because the expected value (or mean) of Y is the probability that Y=1 and, therefore, is limited to the range 0 through 1, inclusive. For details see [7]. More often in clinical trials, the status of a patient is assessed by the presence or absence of a disease for instance, the trial may focus on whether a treatment is effective or non-effective in treating a particular disease.

In our model formulation, Y_j indicates whether there is a recurrence of malaria or not on participant j in the clinical study. We write $Y_j = 1$, if there is no first recurrence of malaria in the patient (that is, a treated child stays malaria free within a given follow-up period) and $Y_j = 0$, if there is recurrence of the disease (that is, treatment failure). If we let π_j denote the probability of a success on the event of interest on participant j, that is, $\pi_j = P(Y_j = 1),$ then the ratio $\pi_j/(1-\pi_j)$ can take on values between 0 and plus infinity. Furthermore, the natural logarithm (ln) of $\pi_j/(1-\pi_j)$ can take on values between minus infinity and plus infinity. We assume that the probability of no recurrence of the disease depends on some covariates x_{ij} and the relationship between this probability and the covariates can be described through a logistic model given by,

$$ln\left[\frac{\pi_j}{1-\pi_j}\right] = \beta_0 + \beta_1 x_{1j} + \beta_2 x_{2j} + \cdots + \beta_k x_{kj}, \quad (1)$$

where β is a $(k+1)$–dimensional parameter vector for $j = 1,2,\ldots N$.

Using the logit transformation, we have

$$\pi_j = \frac{\exp(\beta_0 + \beta_1 x_{1j} + \beta_2 x_{2j} + \cdots + \beta_k x_{kj})}{1 + \exp(\beta_0 + \beta_1 x_{1j} + \beta_2 x_{2j} + \cdots + \beta_k x_{kj})}. \quad (2)$$

We have decided to use the logit link given by equation (2). An alternative might have been to use the logarithmic link $(\pi_j = 1 - \exp(\beta_0 + \beta_1 x_{1j} + \beta_2 x_{2j} + \cdots + \beta_k x_{kj})$, which should correspond to the use of the relative risk instead of the odds ratio. However, the probability of a no recurrence of malaria is quite high for some covariates, which makes this choice inappropriate. An increase from 0.09 to 0.1 is quite different from an increase from 0.9 to 1.0.

In the model literature, it is sometimes said that the number of covariates should not exceed 1/10 of the number of successes (or failures). In our final models we never had more than four independent variables,

even though we had two or three more in some models, which were later rejected. This is clearly less than 1/10 of the number of successes.

During the whole period we observed 104 recrudescence cases out of 178 in Konde and 95 out of 206 in Uzini. Of these recrudescence cases 87 and 80 respectively, occurred during the first 42 days. The number of recrudescence during the second period is sometimes smaller than ten times the number of covariates but we want to keep the same models for all periods.

3.1. Description of Covariates

During the trial period due to administrative reasons and other complications, information on all covariates was not possible for all participants. We consider only those with complete data on the variables of interest which are to be included in the model, that is, 142 patients in Konde and 193 in Uzini.

The background variables (covariates) observed during the trial were:

1. Time: The date of enrolment of the children into the study in calendar days was known in Konde. In Uzini the exact day was not given but since the children were numbered consecutively after recruitment day we used the id-number as a proxy. Time was included as a potential variable since it could explain changes in the weather and the abundance of mosquitoes and is measured by calendar days after start of the study.

2. Age: The age of the children recruited for the study. The age of each patient was given in months. All children that participated in the study were less than five years and the youngest child was one month. This variable was only measured in Uzini.

3. Drug Type: The treatment administered during the trial. This was artesunate plus sulfadoxine-pyrimethamine (ASP) or sulfadoxine-pyrimethamine (SP). We have chosen *DRUG* = 1 and *DRUG* = 0, as codes for the covariates ASP and SP, respectively.

4. Resistance genes: Three positions, which are known to covary with resistance to treatment studied. These are called 1(*pdhfr* 51), 2 (*pdhfr* 59) and 3 (*pdhfr* 108). For each patient this was noted down as a three letter sequence. For example RSR means R is on position 1, S, on position 2 and R again is on position 3. If in the blood sample there were both parasites with the resistant and the sensitive gene at a position this was indicated by an M. For example RMM means that all parasites had a resistant gene at position 1, but that there were both parasites with resistant and sensitive genes at positions 2 and 3. These alphabetical sequences were transformed into numerical variables to be used in the analysis in two alternative ways:

a) M´, R´, or S´: The total number of M', R' and S', respectively in the sequence. For example, RSR gave the variables M´=0, R´=2 and S´=1.

b) M'_i, R'_i, or S'_i: Binary indicators telling if the letter at position *i* was an M, R or S. For example, RSR gave the variables $M'_1 = 0$, $R'_1 = 1$, $S'_1 = 0$; $M'_2 = 0$, $R'_2 = 0$, $S'_2 = 1$; $M'_3 = 0$, $R_3' = 1$, $S'_3 = 0$

We note that these variables are linearly related (e.g. M´ + R´ + S´ = 3), which means that only a subset can be used simultaneously in the equations.

5. Parasite density: The number of parasites per millilitre of blood on Day 0, the date of entry of the patient into the study. This variable was denoted D0p. The ranges for this number were 51 – 6200 and 50 – 5982 parasites per millilitre of blood in Konde and Uzini, respectively.

3.2. Handling Partial or No Information on some Covariates

As explained in Section 2.2, some children enrolled were removed during the trial period due to administrative problems or other complications. There were also laboratory problems leading to a missing genotyping for one of the follow-up times. This resulted in unobserved or incomplete information on these children. We had complete data for 142 (out of 178) children in Konde and 193 (out of 206) in Uzini. In percentage, at least one data variable is missing for 20% and 6%, respectively. In our opinion the administrative reasons for lost to follow-up information were independent of the outcome of the study. We assume that participants with incomplete data do not contribute to the analyses. In other words, data is missing at random.

In addition to the missing information as a result of the above reasons, there were some covariates that

were measured either in Konde only or in Uzini only and not in both treatment centres. When this was the case, we made some assumptions or adjustments before applying models to data.

Information on date of recruitment into the study was available in the data from Konde only. The date was not available in the data from Uzini. Instead, we used each patient's identification number, which was given to each patient following the order of recruitment into the study.

The covariate AGE was available only in the data set from Uzini. We only applied the model with age as one of its covariate to this data set. As concerns the variable D0p, after some preliminary analysis, it was observed that the number of parasites per mililitre of blood in patients on date of entry into the study was quite skewed and therefore we decided to dichotomize using the variable PCODE. Here PCODE (parasite code) = 0 if number of parasites per millilitre of blood is less than 100 and PCODE = 1, if the number is greater than or equal to 100.

4. SOME KEY SCIENTIFIC HYPOTHESES

Logically, if the recurrences of malaria were mainly due to reinfection from new mosquito bites, the time until next reinfection should depend on the abundance of mosquitoes. The amount of mosquitoes should in turn depend on the weather (e.g. wet season means more mosquitoes and sunny weather means less clothes). The weather is likely to vary and be reflected in how the reinfection rate varies over time. On the other hand if the recurrences were mostly recrudescence due to the fact that not all parasites were killed, the reinfection rate should not depend on time. We thus formulated the hypothesis that there should be no effect of time.

The risk of disease seems to hinge upon both the age of the host and the intensity of exposure to the parasite. In areas of high endemicity, the prevalence and density of *P. falciparum* parasitemia and the incidence of overall fevers and of malaria-associated fevers increase with age for the first 6 months of life and then gradually decline [8]. The longer children are exposed and the more bites they get; there is possibility of a greater chance of acquiring immunity against malaria. Immunity to severe malaria is acquired after one or two bites [9]. In endemic areas, increasing age is associated with greater antimalarial immunity and improved therapeutic response but there seem to be no general consensus as to how quickly this immunity is acquired [10]. The recurrence rate should be smaller for ASP since the artemisinin derivative with a short half-life rapidly reduces the parasite density while sulfadoxine-pyrimethamine with a longer half-life clears the remaining parasites. The smaller the more sensitive genes there are (i.e. with R) and since an M indicates more parasites, the recurrence rate should be higher for M than for R (which in turn was higher than for S).

A higher parasite load at the time of treatment may be and not conclusively be associated with less favorable outcomes [11]. For D0p two mechanisms may be possible. High values may mean that more parasites have to be killed which could mean that there may be more of them left after treatment. Another argument is that the amount of parasites in blood varies much over time depending on the life cycle of the malaria parasite. A low number means that there are many parasites in a phase which is difficult to treat. That would mean that the recurrence risk is higher for low values on D0p. The last argument most probably is the correct one.

5. PROPOSED MODELS

Before settling down to more specific models, we first looked at some preliminary models with all the covariates. The first model (3) was used to study which combinations of M, R, and S seemed to best explain the outcome. Two other models (4) and (5) respectively, were used to test whether the outcome varied with time and age of the children.

We start up with the following models, and for simplicity of notation we ignore j :

$$\begin{aligned}\log\mathrm{it}(\pi) &= \beta_1 + \beta_2 DRUG + \beta_3 M + \beta_4 R + \beta_5 M'_1 + \beta_6 M'_2 \\ &+ \beta_7 M'_3 + \beta_8 R'_1 + \beta_9 R'_2 + \beta_{10} R'_3\end{aligned} \tag{3}$$

$$\begin{aligned}\log\mathrm{it}(\pi) &= \beta_1 + \beta_2 log(\mathrm{D0}p) + \beta_3 TIME + \beta_4 PCODE \\ &+ \beta_5 DRUG + \beta_6 M' + \beta_7 R'\end{aligned} \tag{4}$$

$$\begin{aligned}\log\mathrm{it}(\pi) &= \beta_1 + \beta_2 log(\mathrm{D0}p) + \beta_3 AGE + \beta_4 PCODE \\ &+ \beta_5 DRUG + \beta_6 M' + \beta_7 R'\end{aligned} \tag{5}$$

where *PCODE* = 0, if number of parasites/mL < 100 and 1, if number of parasites/mL > 100; *DRUG* = 1, if drug is ASP and 2, if drug is SP;

M'_i, and R'_i indicate whether resistant or sensitive genes are present at site i,i = 1,2,3.

5.1. Preliminary Results on Proposed Models

We started with model (3) with M′ and R′, or S′ being the number of sites with either M, R. M'_i, R'_i and S'_i indicating whether resistant or sensitive genes are present at site *i,i* = 1,2,3. The results were not significant, since *p*-values were quite large. This is probably due to the fact that the number of variables becomes too large for data sets of this size.

Considering model (4), in Konde, Time was not significant for all the periods considered. For instance, the period (0 – 84) days, the *p*–value was 0.31. It was the same conclusion in case of Uzini, $p = 0.68$.

The Uzini data set had information on the age of each child that took part in the study. Applying model (6), the analysis showed that age is not a significant factor to explaining the probability of no first recurrence of malaria over the given periods.

6. MODELS WITH CLINICALLY IMPORTANT COVARIATES AND RESULTS

The above models (3, 4 and 5) could not adequately explain the probability of no first recurrence of parasites. However, we formulated two reduced models. These models incorporate DRUG, together with M and R or together with S only. In each model, we can either use M′ and R′ together or S′ only since M is a combination of R and S (that is, S is a function of M and R). We drop the variables which are not significant at both Konde and Uzini but making sure we keep clinically important variables. Epidemiologic methodologists advocate including in a model all covariates clinically and intuitively relevant mindless of their statistical significance [7]. In our case DRUG, PCODE, M′, R′ and S′ are clinically and intuitively important. The two models of interest are:

$$\log it(\pi) = \beta_1 + \beta_2 DRUG + \beta_3 PCODE + \beta_4 M' + \beta_5 R' \quad (6)$$

$$\log it(\pi) = \beta_1 + \beta_2 DRUG + \beta_3 PCODE + \beta_4 S' \quad (7)$$

Applying these models to data from Konde and Uzini, we have results presented in the Appendix in Tables **1**, **2**, and **3** for the periods (0 – 42), (42 – 84) and (0 – 84) days respectively. The log odds (or logits) do not provide an intuitively meaningful scale to interpret the change in the dependent variable. The usual practice is to take the exponent of the log odds which allows interpretation of the coefficients in terms of odds ratios. The log odds ratio estimates presented in these Tables are accompanied by their 95% confidence intervals, respectively.

We interpret the results of Konde for the period (0 – 42) days resulting from model (6), regardless of whether the coefficients are significant or not. The coefficient for the covariate DrugSP is –0.152. This means the log-odds of no recurrence of malaria within this period 0.152 lower for children receiving SP than for those taking ASP as treatment. The odds-ratio is 0.859 meaning if we hold the other covariates at a fixed value, the odds of no first recurrence of malaria (that is, staying free from the disease) for SP over the odds of no first recurrence for ASP are 0.859. In other words, this shows that the odds of no recurrence are 14.01% higher for ASP than with SP. The corresponding probability (not shown on the Tables), of no recurrence for SP as treatment for this model is 0.462.

Looking at the covariate PCODE, the coefficient is positive and is 0.630. This means the log-odds of no first recurrence within the (0 – 42) days period is 0.630 higher when *PCODE* = 1 than when *PCODE* = 0. This is confirmed by the odds ratio of 1.878. This value says that the odds of not having a recurrence within this period are almost 88% higher if the parasite density in the blood of sick children was more than 100 per millilitre. The corresponding probability is 65.3%.

We consider the variable M′. Its log-odds coefficient value is –0.369. This suggests that the log-odds of no recurrence decreases by 0.369 with a one unit increase in the number of loci classified as M. The inverse odds ratio value is 1.4472, meaning the odds of a recurrence are 44.72% higher for a unit increase in the number of loci with both R and S genes. On the other hand, the odds of a recurrence are 19.05 % higher for a unit increase in the R gene. All the other results for the different periods (in Konde and Uzini) can be interpreted in the same way.

We now explain the results taking cognizance of statistical significance of the coefficient of the covariates. We first look at results obtained by applying model (6) to data. Drug type: SP vs ASP. There is no real significant difference between the two. This is indicated by the fact that in Konde for the periods (0 – 42), $p = 0.66$; (42 – 84), $p = 0.85$ and for (0 – 84), $p = 0.30$. In Uzini, considering these same periods, the *p*–values are 0.07, 0.97 and 0.21 respectively.

In Uzini for the interval (0 – 84), it turns out that a better model is equation (6). The decrease in deviance

is $\chi^2 = 0.23$ with one d.f. and $p = 0.63$. In Konde for the same period and same model (6), the decrease is $\chi^2 = 2.49$ with one d.f. and $p = 0.11$.

Considering the period (42 – 84), the decrease is $\chi^2 = 1.43$ with one d.f. and $p = 0.23$ in Uzini, while in Konde we have $\chi^2 = 1.79$ with one d.f. and $p = 0.18$. For the duration (0 – 42) the decrease is 0.29 and $p = 0.59$ in Uzini and 1.29 with $p = 0.26$ in Konde.

Model (7) has covariates DRUG, PCODE and S'. The number of parasites per millilitre of blood (D0p) of sick children was dichotomized and renamed as PCODE. PCODE is significant in Konde ($p = 0.01$; (0 – 84)) as well as S´ ($p = 0.03$). In Uzini, the picture is different. PCODE is not significant ($p = 0.98$; (0 – 84)) as well as S´ ($p = 0.21$; (0 – 84)). The coefficient of PCODE is weakly positive but that of S´ is negative.

In Konde, a division of the length of the trial into periods (0 – 42) and (42 – 84) each shows that PCODE has a higher effect at the second time period (coefficients are 0.63 and 1.40). These effects are significant. S´ equally has a higher effect at the second time period (coefficients are 0.28 and 0.63) but these effects are not significant. In Uzini, for the same periods, the effects are not significant (coefficients are –0.48 and 0.91). S´ still has slightly higher (though negative) effect at the second period than the first (coefficients are –0.20 and –0.34, respectively).

7. DISCUSSION AND CONCLUSION

The results from our analyses indicate that large number of parasites in the blood increases the chances of getting cured, especially the analysis on Konde data. Two plausible explanations may be that, high level of parasites indicates a more careful treatment which may mean a smaller risk for recurrence or a high level indicates that the malaria is in a phase which is easier to treat. In Uzini we can still say with caution that the higher the number of parasites in the blood, the higher the probability of not witnessing a recurrence of the disease, but on the other hand, the smaller the number of sensitive genes, the smaller the risk of recurrence.

The effect of parasites density on the probability of getting cured was more obvious during the second period. The number of parasites per millilitre is less than 100 means that there is more frequent recrudescence. This is in accordance with the assumption that most first recurrences may be recrudescence. New infections could be more likely to depend on time through changing weather and seasons.

The covariate S´ also has a positive effect that is, the probability of cure is higher when the genes are sensitive (reasonable easier to kill completely). A preliminary analysis showed that time was not significant. This means that the recurrence in malaria is most probably due to recrudescence. The fact that age is not a significant factor for a first recurrence, may mean that there is no effect of partial immunity with age in the area or that the age span is too short. Reappearance of malaria was more associated with the number of M genes followed by the number of R genes, in that order. This is logical since M represents multiple infections and R a single resistant strain. The picture in Uzini is somewhat different. The effect of all covariates was not significant for all whole periods. This may insinuate that recurrence may had only been delayed rather than been stopped. Though the results indicate that no covariate is significant, they however provide the bases for further research. It would also be interesting to test if the covariates have the same effect on recovery probability and on the malaria–free time if there was a recurrence.

In conclusion, the findings that cure was less common for low density parasitemias is somewhat surprising and contradictory to the hypothesis that the higher number of parasites the longer it takes to eradicate all. However a biological hypothesis may be that in those infections the parasites are less actively reproducing and metabolically active and therefore less sensitive to the effect of the drugs. This clearly needs to be further studied. In contrast, the finding that the number of resistant genes reduces the effect of the long acting compounds sulphadoxine - pyrimethamine is clearly as expected since the more mutations associated with tolerance or resistance there are in an infection, the better the chances for the parasites to survive the exposure of the sulphadoxine-pyrimethamine.

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APPENDIX: PARAMETER ESTIMATES FOR TWO CLINICALLY IMPORTANT LOGISTIC REGRESSION MODELS

Table 1: (0 – 42) days: Model 6

ESTIMATES ON KONDE DATA			ESTIMATES ON UZINI DATA		
Parameter	**β**	**95% CI**	**Parameter**	**β**	**95% CI**
Intercept	0.016	(-1.657, 1.696)	Intercept	0.381	(-0.737, 1.546)
DrugSP	-0.152	(-0.837, 0.528)	DrugSP	-0.555	(-1.167, 0.045)
PCODE	0.630	(-0.105, 1.396)	PCODE	-0.483	(-1.423, 0.374)
M′	-0.369	(-0.976, 0.225)	M′	0.266	(-0.147, 0.691)
R′	-0.175	(-0.786, 0.426)	R′	0.365	(0.029, 0.711)
Null dev: 194.03 on 141 df; Resid dev: 188.67 on 137 df(187.01 on 135 df)			Null dev: 256.97 on 192 df; Resid dev: 248.19 on 188 df(242.95 on 186 df)		
(0 – 42) days: Model 7					
Intercept	-0.771	(-1.535, -0.053)	Intercept	1.415	(0.552, 2.380)
DrugSP	-0.115	(-0.793, 0.560)	DrugSP	-0.548	(-1.160, 0.051)
PCODE	0.669	(-0.060, 1.430)	PCODE	-0.495	(-1.433, 0.360)
S′	0.278	(-0.294, 0.867)	S′	-0.338	(-0.670, -0.017)
Residual deviance: 189.96 on 138 degrees of freedom			Residual deviance: 248.48 on 189 degrees of freedom		

Table 2: (42 – 84) days: Model 6

ESTIMATES ON KONDE DATA			ESTIMATES ON UZINI DATA		
Parameter	**β**	**95% CI**	**Parameter**	**β**	**95% CI**
Intercept	0.238	(-2.297, 2.890)	Intercept	-0.063	(-1.460, 1.381)
DrugSP	-0.093	(-1.057, 0.873)	DrugSP	0.018	(-0.803, 0.841)
PCODE	1.397	(0.313, 2.628)	PCODE	0.911	(-0.079, 1.876)
M′	-0.787	(-1.744, 0.072)	M′	-0.013	(-0.582, 0.554)
R′	-0.463	(-1.415, 0.392)	R′	0.273	(-0.213, 0.737)
Null dev: 194.03 on 141 df; Resid dev: 188.67 on 137 df(187.01 on 135 df))			Null dev: 147.32 on 133 df; Resid dev: 142.36 on 129 df		
(42 – 84) days: Model 7					
Intercept	-1.509	(-2.758, -0.439)	Intercept	0.608	(-0.329, 1.597)
DrugSP	-0.145	(-1.097, 0.802)	DrugSP	0.032	(-0.782, 0.851)
PCODE	1.454	(0.386, 2.674)	PCODE	0.842	(-0.135, 1.787)
S′	0.630	(-0.198, 1.562)	S′	-0.201	(-0.645, 0.269)
Residual deviance: 101.65 on 78 degrees of freedom			Residual deviance: 143.79 on 130 degrees of freedom		

Table 3: (0 – 84) days: Model 6

ESTIMATES ON KONDE DATA			ESTIMATES ON UZINI DATA		
Parameter	**β**	**95% CI**	**Parameter**	**β**	**95% CI**
Intercept	-0.312	(-2.196, 1.472)	Intercept	-0.150	(-1.230, 0.925)
DrugSP	-0.432	(-1.263, 0.378)	DrugSP	-0.370	(-0.952, 0.206)
PCODE	1.402	(0.403, 2.595)	PCODE	0.008	(-0.809, 0.813)
M′	-0.848	(-1.548, -0.169)	M′	0.142	(-0.264, 0.554)
R′	-0.516	(-1.179, 0.141)	R′	0.226	(-0.101, 0.562)
Null dev: 158.60 on 141 df; Resid dev: 143.41 on 137 df(142.35 on 135 df)			Null dev: 266.39 on 192 df; Resid dev: 263.27 on 188 df(259.40 on 186 df)		

(Table 3). Continued.

(0 – 84) days: Model 7					
Intercept	-2.265	(-3.483, -1.280)	Intercept	0.480	(-0.318, 1.304)
DrugSP	-0.359	(-1.175, 0.439)	DrugSP	-0.365	(-0.947, 0.210)
PCODE	1.450	(0.462, 2.637)	PCODE	-0.005	(-0.820, 0.797)
S′	0.664	(0.031, 1.307)	S′	-0.204	(-0.526, 0.110)
Residual deviance: 145.9 on 138 degrees of freedom			Residual deviance: 263.50 on 189 degrees of freedom		

REFERENCES

[1] Mårtensson A, Ngasala B, Ursing J, *et al*. Influence of consecutive-day blood sampling on polymerase chain reaction-adjusted parasitological cure rates in an antimalarial-drug trial conducted in Tanzania. The Journal of Infectious Diseases 2007; 195(4): 597-601. https://doi.org/10.1086/510910

[2] Okell LC, Cairns M, Griffin JT, *et al*. Contrasting benefits of different artemisinin combination therapies as first-line malaria treatments using model-based cost-effectiveness analysis. Nature Communications 2014; 5. https://doi.org/10.1038/ncomms6606

[3] Yavo W, Konaté A, Kassi FK, *et al*. Efficacy and safety of Artesunate-Amodiaquine versus Artemether-Lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in sentinel sites across Côte D'Ivoire. Malaria Research and Treatment 2015; 2015. http://dx.doi.org/10.1155/2015/878132

[4] Kum CK, Thorburn D, Ghilagaber G, Gil P, Björkman A. A nonparametric bayesian approach to estimating malaria prophylactic effect after two treatments. International Journal of Statistics in Medical Research 2013; 2(2): 76-87. http://dx.doi.org/10.6000/1929-6029.2013.02.02.01

[5] Kum CK, Thorburn D, Ghilagaber G, Gil P, Björkman A. On the effects of malaria treatment on parasite drug resistance–probability modelling of genotyped malaria infections. The International Journal of Biostatistics 2013; 9(1): 135-48. https://doi.org/10.1515/ijb-2012-0016

[6] Mårtensson A. PCR adjusted cure rates in clinical trials of antimalarial drugs in Africa: Influence of extended follow-up and consecutive day blood sampling. PhD thesis, Institutionen för medicin/Department of Medicine 2008.

[7] Hosmer Jr DW, Lemeshow S, Sturdivant RX. Applied Logistic Regression. John Wiley & Sons 2013. https://doi.org/10.1002/9781118548387

[8] Doolan DL, Dobaño C, Baird JK. Acquired immunity to malaria. Clinical Microbiology Reviews 2009; 22(1): 13-36. https://doi.org/10.1128/CMR.00025-08

[9] Gupta S, Snow RW, Donnelly CA, Marsh K, Newbold C. Immunity to non-cerebral severe malaria is acquired after one or two infections. Nature Medicine 1999; 5(3): 340-3. https://doi.org/10.1038/6560

[10] Griffin JT, Hollingsworth TD, Reyburn H, Drakeley CJ, Riley EM, Ghani AC. Gradual acquisition of immunity to severe malaria with increasing exposure. Proceedings of the Royal Society of London B: Biological Sciences 2015; 282(1801): 20142657. http://dx.doi.org/10.1098/rspb.2014.2657

[11] Ittarat W, Pickard AL, Rattanasinganchan P, *et al*. Recrudescence in artesunate-treated patients with falciparum malaria is dependent on parasite burden not on parasite factors. The American Journal of Tropical Medicine and Hygiene 2003; 68(2): 147-52. https://doi.org/10.4269/ajtmh.2003.68.147

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