Analysis of vaccine efficacy under time dependent variation

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Analysis of Vaccine Efficacy Under Time Dependent Variation

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Certification

This is to certify that this research work was carried out by **Ali Mohamed Ali** under our supervisions.

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Dedication

This work is dedicated to my lovely wife Bimmanga Suleiman and my son Mohamed Ali. Thank you for being patient for the duration of my studies, you have gone through a difficult time raising our child while I am way during all this time. When there was no hope you gave me hope, when I cried for being away from you, you encouraged me with sweet words of hope for our life. You are the best to me and I will always be the best to you. Thanks to my son for being a good boy to your mother during my two years of absence. I left you when you were only three months old. Your mother told me that you never stopped yelling for baba (father). You missed me a lot at the very important time of your life.

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List of Abbreviations

ADI: Active Detection of Infection

AIC: Akaike's Information Criterion

ATP: According to Protocol

BDH: Bagamoyo District Hospital

DTPw: Diphtheria, Tetanus, whole-cell Pertussis

GSK: GlaxoSmithKline

HbsAg: Hepatitis B Surface Antigen

HBV: Hepatitis B Virus

HIV: Human Immunodeficiency Virus

ITT: Intention to Treat

PCD: Passive Case Detection

PH: Proportional Hazard

RTS: Hybrid protein comprising Hepatitis B surface antigen and Circusporozoite protein of *P.falciparum*

RTS,S: Particulate antigen, containing both RTS and Hepatitis B surface antigen proteins

SBC: Schwarz Bayesian Criterion

US: United States

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Abstract

In this phase IIB, double-blind, single center, controlled trial involving 340 eligible infants, conducted between July 2006 and January 2009 in Bagamoyo, Tanzania, 340 infants were randomly assigned to receive three doses of either study vaccine (RTS,S) or Hepatitis B vaccine at 8, 12, and 16 weeks of age.

For each infant, blood slide for malaria parasitemia was taken and fever was recorded during the active (biweekly blood draws to check for *plasmodium falciparum* parasites) and passive surveillance periods. Children were removed from the active detection of infection surveillance once they became infected. The objective was to apply time to event methodology to assess the efficacy of malaria vaccine against febrile malaria.

Cox model was implemented to estimate the vaccine efficacy, which was defined as hazard rate reduction (1 – Hazard rate). Cox model was adopted since the model can handle censored data, which is not the case for other type of regression model.

Different residuals were used for examining different aspects of the Cox model, and the model used was seen to be appropriate.

A total of 60 new febrile malaria infection cases, 34 in the Hepatitis B group and 26 in the RTS,S group, were observed in the period from 14 days post dose 3 to the end of follow-up period. The incidence of febrile malaria was 0.19 and 0.14 per person year for Hepatitis B and RTS,S, respectively. During 17.5 months of follow up period, which started 14 days after third dose of vaccine, the hazard rate reduction for the study vaccine against febrile malaria infection was 33% (95% CI, -11.10 to 60.10; p-value=0.1256).

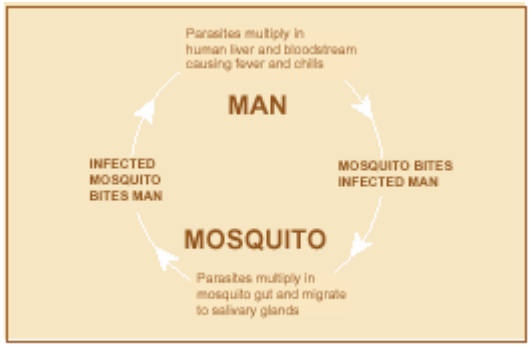
Given the limited sample size and number of new malaria case, the analysis didn't have enough power to show the efficacy of the RTS,S against febrile malaria infection over 17.5 months of follow-up period.

Key Words: Febrile malaria, Survival, Proportional hazard model, Vaccine efficacy.

1.0 Introduction

Malaria is an infectious disease caused by a parasite, *Plasmodium,* which infects red blood cells.[33] There are four identified species of *Plasmodium* causing human malaria, namely *Plasmodium malariae, Plasmodium ovale, Plasmodium falciparum*, and *Plasmodium vivax.* Most of malaria disease cases in Africa are due to *Plasmodium falciparum.* Malaria symptoms appear about 9 to 14 days after the infectious mosquito bite, although this varies with different plasmodium species. Typically, malaria produces fever, headache, vomiting, and other flu-like symptoms.

The malaria cycle involves two hosts, humans and *Anopheles* mosquitoes. The disease is transmitted to humans when an infected *Anopheles* mosquito bites a person and injects the malaria parasites (sporozoites) into the blood. Sporozoites travel through the bloodstream to the liver, mature, and eventually infect the human red blood cells.[26]



Man and mosquito play complementary roles in the malaria cycle.

Every year, malaria kills between 1 and 3 million people and infects 300-500 million. Malaria is the leading cause of death among sub-Sahara African children under the age of five. In addition to its human toll, malaria costs Africa US $12 billion per year and up to 40% of the government health spending. At current, the number of deaths caused by malaria in Tanzania is estimated to equal around 80,000.[27]

Progress has been made in controlling malaria by introducing insecticide-treated nets[21] and highly effective artemisinin-based combination treatments.[22] There is evidence that the incidence of malaria is decreasing in some areas.[6,23,24] These advances have renewed interest in the prospects for the control of malaria and even its elimination in areas in which *P. falciparum* was previously endemic.[14] A safe and affordable vaccine providing protection against malaria would be an important addition to control strategies and should be assessed in the context of the use of insecticide-treated nets and the availability of artemisinin-based combination treatments.[5] Because severe morbidity and death due to *Plasmodium falciparum* disproportionately occurs in infants and young children living in sub-Saharan Africa, this target population has been the principal focus of malaria vaccine development.[4]

However, the development of a safe and effective malaria vaccine remains an urgent unmet medical need for vast populations living in malaria-endemic regions.

The candidate pre-erythrocytic malaria vaccine RTS,S (GlaxoSmithKline (GSK), Rixernsart, Belgium), targets the circumsporozoite protein and has been evaluated in combination with two different adjuvant systems: AS01 and AS02. Clinical development of RTS,S in field trials began with the AS02 adjuvant system.[5]

The malaria vaccine candidate RTS,S, formulated with the adjuvant system AS02, specifically targets the pre-erythrocytic stage of *Plasmodium falciparum* parasite and cannot be used for other malaria species.

The vaccine was first tested in infants in Mozambique and showed a promising safety profile. It appeared to be immunogenic and conferred about 62% protection against malaria infection in infants. In this trial doses of RTS,S and Diphtheria, Tetanus, whole-cell Pertussis (DTPw) were given 2 weeks apart[3].

In the current trial, doses of RTS,S were given together with standard DTPw vaccine in a population of 6-12 months old babies. RTS,S conferred 65% protection against infection over the first 9 months of the trial and 43% protection against febrile malaria defined as 500 parasite and fever[1].

The current report aims to apply time to event methodology to describe the results of the vaccine of the later mentioned trial by including the information available for 17.5 months of follow-up in infants living in an area of perennial malaria transmission in Tanzania.

1.1 Objective

To apply model for time to event to assess the efficacy of RTS,S/AS02D against febrile malaria infection (defined as 500 *Plasmodium falciparum* asexual parasitemia per µL + fever) in infants immunized with RTS,S/AS02D given at three doses at 8, 12 and 16 weeks of age and followed up for 17.5 months.

2 Description of the data

The data come from a phase IIb randomized, single centre, double-blind, controlled study of the efficacy of RTS,S/AS02D, a candidate malaria vaccine, administered in three doses in infants in Bagamoyo, Tanzania. The subjects were randomized to receive RTS,S or Hepatitis B vaccine (Engerix-B) as control.

Cohorts

The analysis is performed in two cohorts: intention-to-treat (ITT) cohort for efficacy and according-to-protocol (ATP) cohort for efficacy.

The ITT cohort includes all enrolled subjects for whom data concerning the efficacy endpoint measures are available and who received at least one dose of vaccine (RTS,S or Engerix-B).

The ATP cohort includes all enrolled subjects for whom data concerning the efficacy endpoint measures are available, who received all three doses of RTS,S/AS02D or Engerix-B according to randomization list, received clearance drug, and were parasite negative at the start of active detection of infection (ADI).

Case definition

The efficacy endpoint is time to febrile malaria infection. The infections was diagnosed via the passive case detection (PCD) surveillance and by the ADI surveillance (see section 3.2). The case definitions of febrile malaria infection used for defining endpoints, are shown in Table 1.

Table 1: *Case definittion*

Clinical malaria (Primary case definition)	• The presence of *P. falciparum* asexual parasitemia above 500 per μL) on Giemsa stained thick blood films AND • The presence of fever (axillary temperature ≥ 37.5°C)

Time at risk

Time at risk for the ITT cohort started at the day of the first vaccination, while for the ATP cohort it started 14 days post dose 3 (RTS,S or Engerix-B).

The time at risk ended whenever any of the following conditions was fulfilled: the case definition for febrile malaria was observed; loss of follow up; emigration from the study area; withdrawal; death; end of the follow-up period.

Covariates:

Malaria transmission depends on many factors. The estimation of vaccine efficacy should take into consideration the impact of these factors to remove potential confounding and increase the precision. Therefore, the following covariates will be considered in modelling process:

Distance from Bagamoyo District Hospital (BDH (in kilometers)): continuous variable

Village of residence: categorical variable (Table 2 explains the area composition, Figure 2 below shows the villages present in the study areas)

Table 2: *Area categorization*

Area category	Villages
Area 1	YOMBO,MATIMBWA AND CHASIMBA
Area 2	KIROMO,BUMA AND MATAYA
Area 3	MLINGOTINI,MATUMBI,ZINGA,MAPINGA AND KEREGE
Area 4	PANDE AND KONDO
Area 5	MAGOMENI,DUNDA AND KAOLE

3.0 Methods

3.1 Study area and design

This phase IIB, single centre, double-blind, controlled trial was conducted between July 2006 and January 2009 by the Bagamoyo Research and Training Centre, a branch of the Ifakara Health Institute in Bagamoyo, Tanzania. The study was conducted in and around Bagamoyo town, on the Tanzanian Coast.

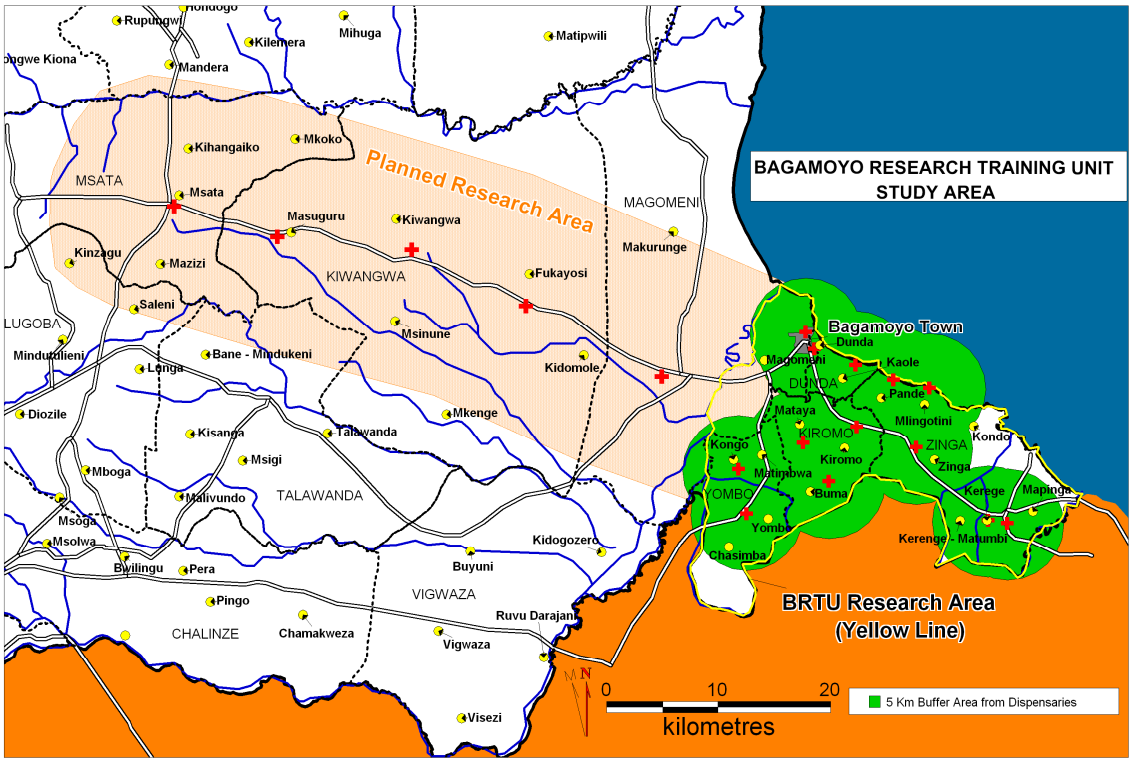


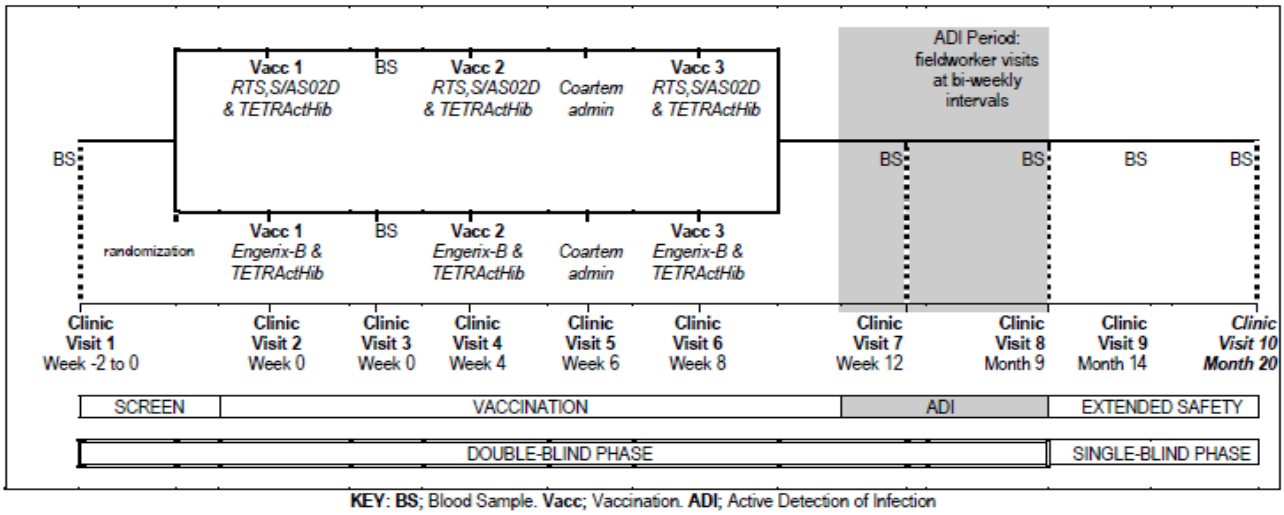
Figure 1*: Map of the BRTC study area. Green denotes initial study area, light brown highlights the recent Msata expansion within Bagamoyo District*

The study area (Figure 1) covered about 1160 square kilometers. The eastern border of the study area was formed by the Indian Ocean, with the Ruvu River forming part of the western and northern borders.

Informed consent in Swahili was obtained from resident pregnant women in their third trimester. Consenting women were counseled and screened for Human Immunodeficiency Virus (HIV) and hepatitis B (HB). HIV-positive women were referred to Bagamoyo District Hospital (BDH) for management as per National guidelines and were excluded from the study. Women who tested positive for Hepatitis B Surface Antigen (HbsAg) were referred to the herpetologist/gastroenterologist at Muhimbili National Hospital for further investigations and symptomatic treatment. If there were no symptoms and signs of chronic liver disease, they were discharged home and invited to deliver at BDH. The first dose of the active hepatitis B Virus (HBV) vaccination for the newborn was given within 12 hours after delivery. The consented infants were screened to enter the study using pre-defined inclusions and exclusions criteria provided by the (GSK) manufacture of vaccine.

3.2 Procedures

Figure 2 presents the trial design and the follow-up scheme. Three hundred forty eligible infants were randomly assigned to receive three doses of either study vaccine (RTS,S) or Hepatitis B vaccine (Engerix-B). The infants, who were assigned to the malaria vaccine, received RTS,S/AS02D on the left enter lateral thigh. Each infant was scheduled to receive three doses of either vaccine, at 8 (first dose), 12 (second dose) and 16 (third dose) weeks of age. After each vaccination, infants were observed for one hour for monitoring of any adverse events. Trained field workers visited the infants at home every day for the next 6 days to record local and general adverse events. The mother/guardian was directed to visit to hospital whenever their baby felt sick (PCD surveillance). The PCD surveillance started from the day of the first dose to the end of the study period. During this phase, blood slide for malaria was taken and fever was recorded when infants attended hospital. ADI started from 14 days post dose three to 9 months post dose one. During this phase, infants were followed at home biweekly by a trained field worker and each time blood slide for malaria parasitemia and fever was recorded, and the child was removed from ADI surveillance once they became infected. Each infant was followed up for 20 months.



KEY: **BS**; Blood Sample. **Vacc**; Vaccination. **ADI**; Active Detection of Infection

Figure 2: *Trial design*

4.0 Statistical Methods

4.1 Exploratory Data Analysis

In order to get insight of the data, the summary statistics, tables of frequencies and Kaplan-Meier estimator for survival function were considered.

4.1.1 Kaplan-Meier Estimator for Survival Function

The product-limit estimator proposed by Kaplan-Meier was used to estimate the survival distributions of the RTS,S group and Engerix-B group. The product-limit estimator is a step function with a jump at the observed event times; it provides an efficient means of estimating the survival function for right-censored data. The main assumption for the Kaplan-Meier estimator is that potential censoring time is unrelated to the potential event time.[17] In our analysis we plotted the Kaplan-Meir curves for the cumulative incidence of febrile malaria cases for RTS,S and Engerix-B.[25]

Log-rank[32] test is a non-parametric hypothesis test used to compare the survival 1distributions of two samples, accommodating censored data. The null hypothesis being tested is that there is no overall difference between the two survival curves. For two groups comparison, under this null hypothesis, the distribution of the log-rank test statistic is approximately chi-squared with one degree of freedom.[19] The log-rank test was then employed to evaluate whether or not the survival curves for the RTS,S and Engerix-B were statistically similar.

Because the Kaplan-Meier estimator does not adjust for the effect of covariates, the data were additionally analyzed by using the Cox model (Cox 1972).

4.2 Proportional Hazard Model (Cox Model)

In this report, we focused on estimating vaccine efficacy against febrile malaria. Different measures have been proposed to measure the vaccine efficacy (VE). A possible measure is VE = 1 – RR, where RR is a measure of relative risk in the vaccinated group compared with the unvaccinated or placebo group. Others measures are using attack rate or using hazard rate. In our report we will use hazard ratio to estimate vaccine efficacy. Thus, vaccine efficacy is defined as a hazard rate reduction, i.e., 1-HR, where HR is the hazard ratio. The hazard ratio was estimated by using the Cox model.

Time-to-event analysis provides a method to include patients who fail to complete the trial (censored data) The model does not require assumptions about the parametric distribution of the survival times. Therefore, it is more robust. However, the price to pay is a loss of precision in the estimated predictor effects.[35]

Generally, the proportional hazards model can be expressed in the form

$$h_i(t) = \exp(\beta' x_i) h_0(t),$$

where $h_i(t)$ is the hazard function for the ith individual, for whom a set of value of explanatory variables $\mathbf{x} = (x_1, x_2, \ldots, x_p)$' were measured.

In our analysis, we first considered the Cox model, which included only the treatment as a covariate in order to evaluate the efficacy of the study vaccine when no any other factor present. The model is:

$$h_i(t) = \exp(\beta x_i) h_0(t)$$

In order to correct for possible confounding effects, the model was further extended to include the distance from BDH to the infant's residence and the area where the infant live. Thus, the hazard function for the ith infant in the study vaccine $h_i(t)$ was modelled as follows:

$$h_i(t) = \exp(\beta_1 x_i + \beta_2 Area_{1i} + \beta_3 Area_{2i} + \beta_4 Area_{3i} + \beta_5 Area_{4i} + \beta_6 Dis\tan ce_i) h_0(t)$$

where $h_i(t)$ is the hazards for first febrile malaria infection at time t, for the i^{th} infant and $h_0(t)$ is the baseline hazard and

$$X = \begin{cases} 1 \text{ if infant receives RTS, S} \\ 0 \text{ if infant receives Engerix - B} \end{cases}$$

$$Area_1 = \begin{cases} 1 \text{ if infant live in area 1} \\ 0 \text{ if otherwise} \end{cases} \qquad Area_2 = \begin{cases} 1 \text{ if infant live in area 2} \\ 0 \text{ if otherwise} \end{cases}$$

$$Area_3 = \begin{cases} 1 \text{ if infant live in area 3} \\ 0 \text{ if otherwise} \end{cases} \qquad Area_4 = \begin{cases} 1 \text{ if infant live in area 4} \\ 0 \text{ if otherwise} \end{cases}$$

4. 3 Assessment of model adequacy

Model-based inferences depend completely on the fitted statistical model. For these inferences to be valid in any sense of the word, the fitted model must provide an adequate summary of the data upon which it is based.[15]

We are interested in examining four aspects of the proportional hazard model fitted, which will be explained in the subsequent sections. Different residuals were used for examining different aspects of the Cox model. The scaled Schoenfeld were discussed in section 4.3.1. These residuals were used for checking the proportional hazard assumption. In section 4.3.2, the notion of a martingale residual was presented. These residuals were useful for determining the function form of a covariate to be included in the proportional hazard model. The deviance residuals were discussed in section 4.3.3. These residuals were used for checking the overall fit of the final model. In section 4.3.4, we examined the influence each subject has on the model fitted.

4.3.1 Scaled Schoenfeld Residuals

The Cox model makes assumption of proportionality of the hazard, which has to be checked before the model application. In this section, the scaled Schoenfeld residuals methodology will be discussed.

Schoenfeld residuals for the Cox model are defined for each predictor variable in the model[8].

The i^{th} Schoenfeld residual for X_j, the j^{th} explanatory variable in the model, is given by

$$r_{Pji} = \delta_i \left(x_{ji} - \hat{\alpha}_{ji} \right),$$

Where: x_{ji} is the value of the j^{th} explanatory variable, $j = 1, 2, \ldots, p$, for the i^{th} individual.

$$\hat{\alpha}_{ji} = \frac{\sum_{l \in R(t_i)} x_{jl} \exp(\hat{\beta}' x_l)}{\sum_{l \in R(t_i)} \exp(\hat{\beta}' x_l)}, \qquad \delta_i = \begin{cases} 1 \text{ if uncensored observation} \\ 0 \text{ if censored observation} \end{cases}$$

and $R(t_i)$ is the set of all individuals at risk at time t_i.

Grambsch and Therneau (1994) show that scaled Schoenfeld residuals can be of a great use in diagnostics of Cox regression models, especially in assessing the proportional hazards assumption.

The scaled Schoenfeld residuals are Schoenfeld residuals adjusted by the inverse of the covariance matrix of the Schoenfeld residuals. The null hypothesis for the test on proportional hazards based on the scaled Schoenfeld residuals is that the slope of Schoenfeld residuals against a function of time is zero for each predictor variable. Testing the time dependent covariates is equivalent to testing for a non-zero slope in a generalized linear regression of the scaled Schoenfeld residuals on functions of time.[8]

The *Scaled Schoenfeld residuals*, r^*_{Pi}, are defined as

$$r^*_{Pi} = r\,\mathrm{var}(\hat{\beta})r_{Pi}$$

where r is the number of events among the n individuals, $\mathrm{var}(\hat{\beta})$ is the variance-covariance matrix of parameter estimates in the fitted Cox regression model, and $r_{Pi} = (r_{P1i}, r_{P2i,,,,,,}r_{Ppi})'$ is the vector of Schoenfeld residuals for the i^{th} individual.

Since our primary focus is mainly on the treatment effect and its possible change over time, we therefore applied scaled Schoenfeld residuals only to test time dependent treatment effect.

The graph of the scaled Schoenfeld residuals against the rank of time was first plotted to visually inspect possible patterns.

4.3.2 Determining the Functional Form of Covariate: Martingale Residuals

In this section, we examined the function form to be used for continuous covariate to best explain its effect on survival through a Cox model. The residuals used here called martingale residual.

Martingale residuals can be interpreted as the difference over time of the observed number of events minus the expected number of event under the assumed Cox model, that is martingale residuals are an estimate of the excess number of events seen in the data but not predicted by the model.[17]

In order to investigate whether the correct functional form for the continuous covariates martingale residuals are calculated for the null model and plotted against the values of the covariate(s) and smoothed curve superimposed to aid the interpretation.[12]

The smoothed-fitted curve gives an indication of the function appropriate for the covariate. If the plot is linear, then, no transformation of the covariates is needed.[17]

The i^{th} martingale residual is defined as

$$\hat{M}_i = \delta_i - rc_i$$

Where δ_i take value of 1 if observation is uncensored and 0 if censored and

$$rc_i = \hat{H}_0(Y_i) \times \exp\left(\sum_{j=1}^{m} \hat{\beta}_j \, x_i^{(j)} \right), \; i = 1,. \; . \; . \; , \text{n},$$

where Yi = min(Ti, Ci); T is the event time and C is the censored time. The are the *maximum partial likelihood estimates* and is an empirical estimate of the cumulative hazard at time *t*.

is i^{th} Cox-Snell residual.[31]

In our analysis, we applied martingale residuals to examine the best function of the variable distance to be used in the model. Cox model with no covariate (Null model) was fitted and the martingale residuals were plotted against distance along with a Loess smooth (Cleveland (1979)) was superimposed.

Because martingale residuals are not symmetrically distributed about zero, even when the fitted model is correct, the deviance residuals which are more symmetrically distributed about zero was proposed in the literature for assessing overall goodness of fit.[12]

4.3.3 Assessment of Overall Goodness of fit: Deviance Residuals

In linear models a measure of fit is provided by a quantity known as the deviance.

The deviance is a statistic that is used to summarize the extent to which the fit of a model of current interest deviates from that of the model which is a perfect fit to the data.[12]

Collett (2003) views deviance residuals as martingale residuals that have been transformed to produce values that are symmetric about zero when the fitted model is appropriate.

The residual provides information about how well the model fits each particular observation.

The i^{th} *Deviance residuals*, denoted by D_i is defined as

$$D_i = sign(\hat{M}_i) \times \sqrt{-2\left(\log(\hat{L}_i) - \log(\hat{L}_{si}) \right)}$$

Where $\hat{M}_i$ is the i^{th} *martingale residual*, $\hat{L}_i$ denotes the i^{th} individual's likelihood evaluated at MLE's, and $\hat{L}_{si}$ denotes the i^{th} factor in the saturated likelihood evaluated at the MLE of the parameter.

To assess the effect of a given individual on the model, Klein and Moeschberger.(2003) suggested constructing a plot of deviance residuals versus the risk score, and argued that a potential outlier will have deviance residuals whose absolute values are too large.

In our analysis *deviance residuals* was employed to assess the overall fit of the Cox model. The plot of deviance residuals against estimated risk scores was then examined.

Addition to that, the plot of observed versus expected survival was considered. The Kaplan-Meier curves was used to obtain observed plots for RTS,S and Engerix-B separately, whereas the Cox model containing treatment effect was fitted to obtain expected survival plots.

Because outlying observation tend to influence the effect of the estimated parameter, it was interested in our analysis to investigate the influence of the outlying observation, therefore section 4.3.4 was aimed to explain the identification of influential observation methodology.

4.3.4 Identification of Influential Observation

Conclusions from survival analysis are often framed in terms of estimates of quantities such as the relative hazard and median survival time, which depend on the estimated values of the beta parameters in regression models. Influential observation has the effect that when it is removed from the dataset, the model parameter estimates are increased or reduced by a substantial amount.[12] It is therefore of particular interest to examine the influence of each observation on these estimates.

An approximation to the amount by which $\hat{\beta}_j$ changes when the i^{th} observation is omitted (delta beta), was used in assessing the influential of the given observation to the estimated value. The delta beta was given as:

$$\Delta_i \hat{\beta}_j \approx \hat{\beta}_j - \hat{\beta}_{j\,(i)}, \text{ for i =1,2,..,n.}$$

Where $\hat{\beta}_j$ is the parameter estimates for treatment effect and $\hat{\beta}_{j(i)}$ is the parameter estimates for treatment effect when the i^{th} observation was deleted.

Observation that influence a parameter estimates for treatment effect, will be such that the values of delta beta for these observations, are larger in absolute value than other observation in the dataset.[12] The delta beta was then standardized by dividing the delta beta by the standard error of $\hat{\beta}_j$, and the observed value of this statistic is then compared to the twice standard error of $\hat{\beta}_j$. The observation with value of standardized delta beta

more than twice standard error of $\hat{\beta}_j$ was considered as an influential. And if the observation is influential, the Cox model with and without the observation will be fitted and the results will be compared.

4.4 Model with Time-Dependent Covariate(s)

Because the assessment of the graphical is more subjective, and one may favor the results of his/her interest, the formal procedure for testing the proportional hazard assumption was implemented. In this section time-dependent covariate methods will be discussed.

A time-dependent variable is defined as any variable whose value for a given subject may differ over time. The most important feature of this model is that the proportional hazard assumption is no longer satisfied[12] and so the model can be used as test for proportional hazard assumption. Generally the model with time dependent covariate can be expressed as:

$$h_i(t) = \exp(\beta_1 x_i + \beta_2 x_i * f(t)) h_0(t)$$

where *f*(t) stand for any function of time and $h_0(t)$ is the hazard function for an individual for whom all the variables are zero at baseline, and remain at this same value through time.

Becuase in this study individuals were monitored during the study, and effect of treatment was recorded whose effects may change during the course of the study, a model with time-dependent variable was considered. The suggested function of time proposed by scaled Schoenfeld residuals plot was then studied. The Wald test was used to test the significance of the function of time, by testing if the parameter corresponding to the function of time is significantly different from zero.

5.0 Results

A total of 378 infants were screened, and 340 received the first dose of vaccine. 297 (87.35%) of the 340 children completed the 20 months visits. Table 3 presents the baseline characteristics for both groups. Age distribution at enrollment (first dose) was almost similar in the two vaccine groups, and the mean age was 7.8 weeks.

Table 3: *Baseline characteristics of subjects*

Characteristic	RTS,S/AS02D (n=170)	Engerix-B (n=170)	All subjects
Age			
Age at first dose (weeks)- mean ± sd	7.9 ± 0.8	7.8 ± 0.8	7.8 ± 0.8
Sex- no.(%)			
Boy	85 (50.0)	79 (46.5)	164 (48.2)
Girl	85 (50.0)	91 (53.5)	176 (51.8)
Distance from BDH to home (km) no. (%); cases*			
[0-5[	45 (26.5)	59 (34.7)	104 (30.6); 12*
[5-10[	20 (11.8)	16 (9.4)	36 (10.6); 8*
[10-15[	51 (30.0)	42 (24.7)	93 (27.4); 18*
≥15	54 (31.8)	53 (31.2)	107 (31.5); 35*
Area of residence -no. (%); cases*			
Area 1	30 (17.7)	27 (15.9)	57 (16.8); 29*
Area 2	17 (10.0)	15 (8.8)	32 (9.4); 9*
Area 3	44 (25.9)	58 (34.1)	102 (30.0); 20*
Area 4	14 (8.2)	17 (10.0)	31 (9.1); 5*
Area 5	65 (38.2)	53 (31.2)	118 (34.7); 10*

Percentages may not total 100 because of rounding

5.1 Intention to Treat Cohort

All subjects who received at least one dose of vaccine (RTS,S or Engerix-B) were used in the ITT cohort analysis. During 20 months (from day of first dose to the end of study) of follow up, 73 infants has at least one case of febrile malaria (as defined in Table 1), of which 38 were observed from Engerix-B group and 35 from RTS,S group. This results in

the estimated incidence of febrile malaria of 0.17 and 0.15 per person year, respectively (Table 4).

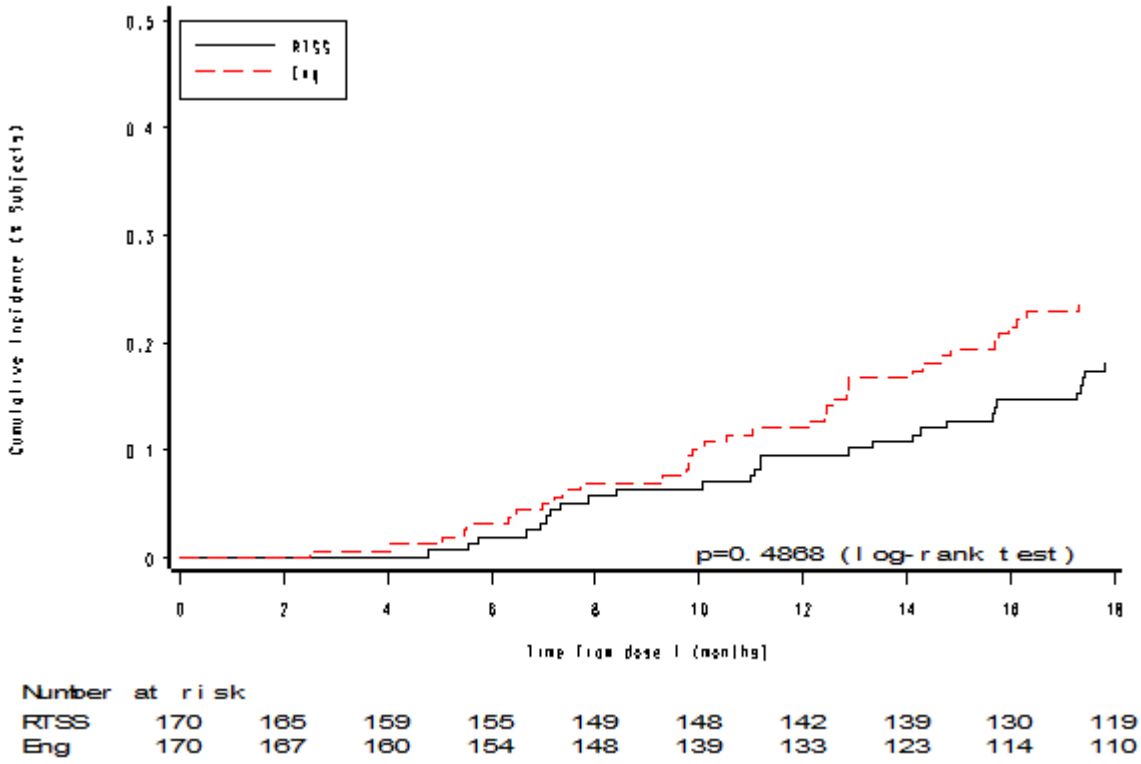


Figure 3: *Kaplan-Meier curves showing the cumulative incidence of at least one malaria case (ITT cohort for efficacy [0-20]) for RTS,S (solid black line) and Engerix-B (doted red line)*

Kaplan-Meier curves (Figure 3) show the cumulative incidence of febrile malaria infection in the two study groups during the entire follow up period. The graph also shows that the Engerix-B group have a constantly higher incidence compared to RTS,S. Very small difference in incidence is observed from day 0 (dose 1) until around 9 months post dose 1, and later the curves start to separate. The separation is not statistically significant (p-value =0.4868). It also observed that until month 9, the incidence of febrile malaria was very small but increased afterwards.

After fitting the Cox model, the estimated hazard ratio was 0.85, and the corresponding hazard rate reduction was estimated to 15.00% (95% CI, -34.60 to 46.40; p-value=0.4870; Table 4).

Table 4*: Vaccine Efficacy. Time to first event (ITT cohort for efficacy [0-20])*

						Point estimate of VE unadjusted for covariates			
Event Type	**Group**	**N**	**n**	**T (year)**	**Incidence rate**	**%**	**LL**	**UL**	**P-value**
Febrile	Engerix	170	38	225.66	0.17	-	-	-	-
Malaria	RTSS	170	35	237.13	0.15	15.00	-34.60	46.40	0.4870

N: number of subjects

n: number of subjects with events

T: Person Years at Risk

VE: Vaccine Efficacy (1-Hazard Ratio)

LL: Lower Limit

UL: Upper Limit

p-value from Cox PH model

Incidence rate=n/T

Table 5 presents parameter estimates for the area of residence and distance from BDH. For one kilometer increase in *Distance*, the hazard of having febrile malaria increased 1.056 times, however, the increase was not statistically significant (p-value= 0.0810). Infants living in area 1 and area 2, respectively have about 4 and 2 times higher hazard of having febrile malaria infection compared to those infants who live in area 5. However, the increase in the hazard for area 2 was not statistically significant.

Table 5*: Vaccine Efficacy. Adjusted for covariates (ITT cohort for efficacy [0-20])*

Effect	**Estimate**	**Standard error**	**P-value**	**Hazard ratio**	LL	UL
Treatment	-0.283	0.237	0.2323	0.754	0.474	1.199
Distance	0.054	0.031	0.0810	1.056	0.993	1.122
Area1	1.403	0.539	0.0092	4.068	1.415	11.695
Area2	0.785	0.525	0.1350	2.192	0.783	6.135
Area3	-0.319	0.677	0.6377	0.727	0.193	2.739
Area4	-0.014	0.657	0.9827	0.986	0.272	3.575

5.2 According to Protocol Cohort

The according to protocol analysis was considered all infants who received all 3 doses of vaccine according to randomization and who were parasite negative at start of ADI. A total of 297 infants, from which 151 were in Engerix-B group and 146 were in RTS,S group, were therefore included in the analysis. After 14 days (2.5 months post dose 1) post dose 3 to end of follow up period (20 months post dose 1), a total of 60 new febrile malaria infection were observed, 34 of 60 from Engerix-B and 26 of 60 from RTS,S, the incidence of febrile malaria was 0.19 and 0.14 per person year for Engerix-B and RTS,S respectively.

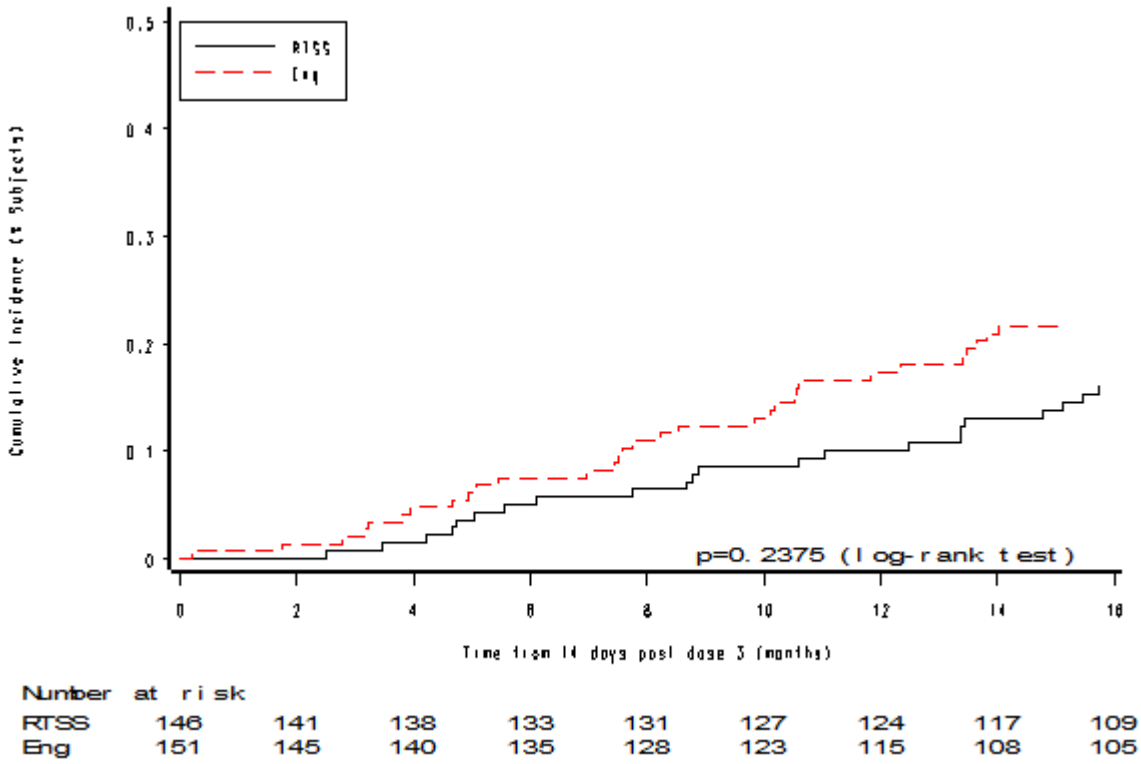


Figure 4*: Kaplan-Meier curves showing the cumulative incidence of at least one malaria case (ATP cohort for efficacy [2.5-20]) for RTS,S (solid black line) and Engerix-B (doted red line)*

Figure 4 shows Kaplan-Meir curves of the cumulative incidence of febrile malaria infection in the two study groups during the entire follow up period. From the graph it can be seen that in Engerix-B group have constantly higher incidence of febrile malaria infection compared to RTS,S. A considerably difference in incidence was observed after 8 months post dose 1, however the difference was not statistically significant (p-value

=0.2375). It also observed that until month 9, the incidence of malaria was very small for both groups but increased thereafter.

The results from fitting the Cox model, gave the hazard ratio of 0.73 and the corresponding hazard rate reduction of 27.0% (95% CI, -22.80 to 56.80; p-value=0.2413; Table 6) over the 17.5 months of follow up period. Adjusted by area of residence and distance to the health centre and community of residence, the estimated hazard ratio was 0.67 and the corresponding hazard rate reduction was equal to 33.00% (95% CI,-11.10 to 60.10; p-value=0.1256; Table 7).

Table 6: *Vaccine Efficacy. Time to first event (ATP cohort for efficacy [2.5-20])*

						Point estimate of VE unadjusted for covariates				Point estimate of VE adjusted for covariates			
Event Type	**Group**	**N**	**n**	**T (year)**	**Incidence rate**	**%**	**LL**	**UL**	**P-value**	**%**	**LL**	**UL**	**P-value**
Febrile	Engerix	151	34	178.56	0.19	-	-	-	-	-	-	-	-
Malaria	RTSS	146	26	181.90	0.14	26.30	-22.80	56.80	0.2413	33.20	-11.10	60.10	0.1256

N: number of subjects

n: number of subjects with events

T: Person Years at Risk

VE: Vaccine Efficacy (1-Hazard Ratio)

LL: Lower Limit

UL: Upper Limit

p-value from Cox PH model

Incidence rate=n/T

The risk of febrile malaria infection was significantly (p-value=0.0317) increased 1.082 times with a unit increase in distance (km) from health centre.

Also, infants living in area 1 and area 2, have about 2 times higher risk of having febrile malaria infection compared to those infants who live in area 5; however the increase is not statistically significant (Table 7).

Table 7: *Vaccine Efficacy. Adjusted for covariates (ATP cohort for efficacy [2.5-20])*

Effect	Estimate	Standard error	P-value	Hazard ratio	LL	UL
Treatment	-0.404	0.263	0.1256	0.668	0.399	1.119
Distance	0.078	0.036	0.0317	1.082	1.007	1.162
Area1	0.894	0.606	0.1402	2.445	0.745	8.024
Area2	0.436	0.561	0.4374	1.546	0.515	4.646
Area3	-0.978	0.788	0.2146	0.376	0.080	1.762
Area4	-0.571	0.738	0.4393	0.565	0.133	2.401

5.3 Assessment of model adequacy

Model check in any application is a crucial step in modeling process and should be done prior the model implementation. Therefore in this chapter we shall present the results of different aspect for the assessment of model adequacy.

5.3.1 Scaled Schoenfeld Residuals

5.3.1.1 Intention to Treat Cohort

Scaled Schoenfeld residuals was implemented to visually observe any possible departure from the proportional hazard assumption. From Figure 5, it can be seen that the smoothed residuals have essentially a slope of zero.

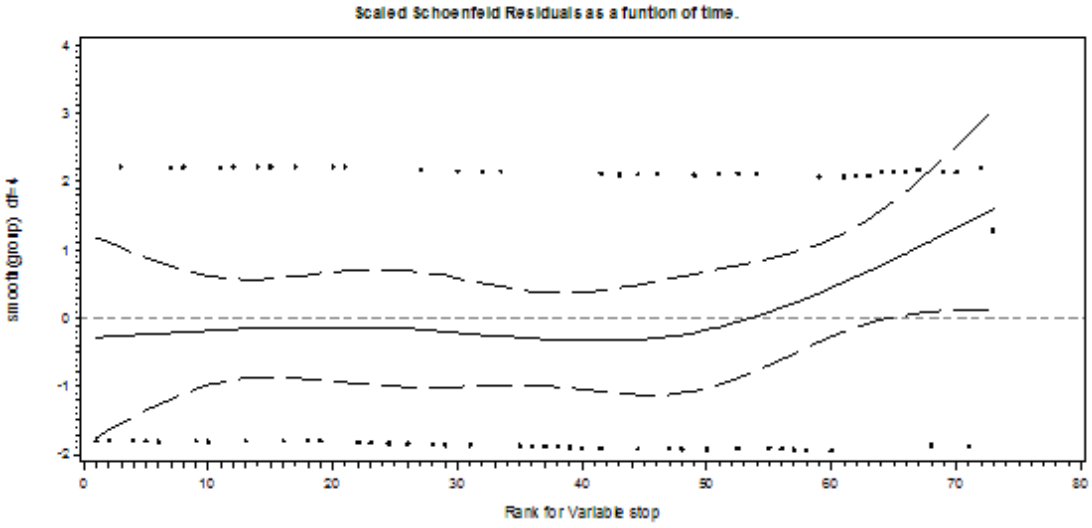


Figure 5: *Schoenfeld residuals plot of residual for treatment group against rank of survival time with a smoothed curve superimposed (ITT cohort for efficacy [0-20])*

5.3.1.2 According to Protocol Cohort

A plot of scaled Schoenfeld residuals against rank of survival time was implemented to visually observe if there was any trend over time. From Figure 6, it was observed that the smoothed residuals were essentially horizontal. The latter observed positive slope is due to the reason that there were more events in the RTS,S group near to the end as compared to Engerix-B group.

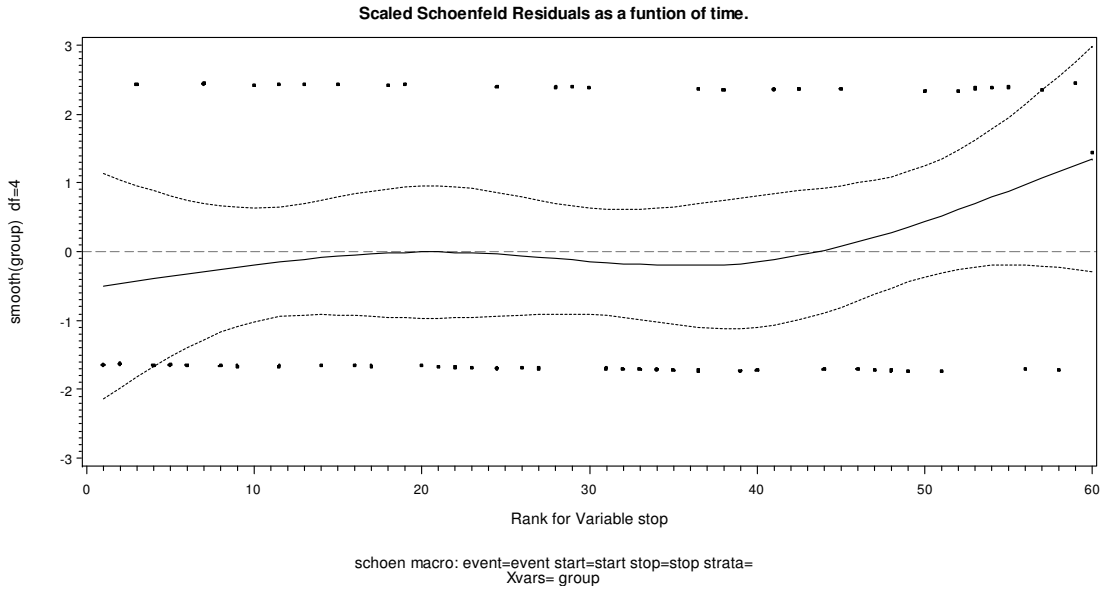


Figure 6: *Schoenfeld residuals plot of residual for treatment group against rank of survival time with a smoothed curve superimposed (ATP cohort for efficacy [2.5-20])*

5.3.2 Determining the Functional Form of a Continuous Covariates

Since in our dataset only distance was continuous variable, therefore martingale residuals was used to Cox regression model fitted to the data. The martingale residuals for the null model were obtained and these were plotted against the corresponding values of the distance from health centre to the subject's home of residence.

5.3.2.1 Intention to Treat Cohort

In Figure 7 (*left panel*), there appears to be a negative curvature for distance between 15 and 25. However, the lines before and after the curvature nearly horizontal. This suggests that using a linear form for distance in the model seems appropriate.

5.3.2.2 According to Protocol Cohort

From Figure 7 (*right panel*), the smoothed curve indicates that the line is nearly horizontal. This show that there is no need for anything other than a linear term in distance.

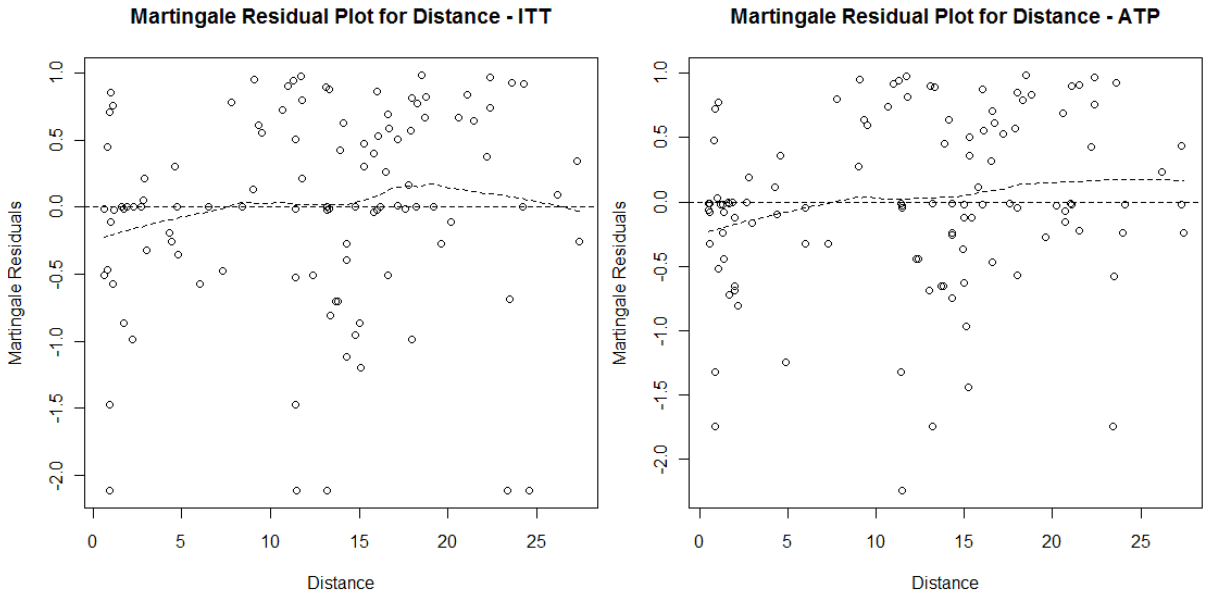


Figure 7: *Martingale residuals for the null model against Distance (ITT-left, ATP-right)*

5.3.3 Assessment of Overall Goodness of fit

Even though the distance does not need to be transformed, to assess the overall goodness of fit is not avoided step. Hence in this part, the results on the assessment of overall goodness of fit will be presented.

5.3.3.1 Intention to Treat Cohort

Upon examining the deviance residual plot (Figure 8 *left panel*), a scatter of random noise about zero was observed. These suggested that the individual fitted well the data, but there was an indication of some outlying observations nevertheless.

Additional to the deviance residual plot, the plot of observed survival from Kaplan- Meir and expected survival from Cox model was employed in assessing the fit of the model used.

Figure 9 (*left panel*) shows the two graphs are very close, this also suggest that the model used fitted the data well.

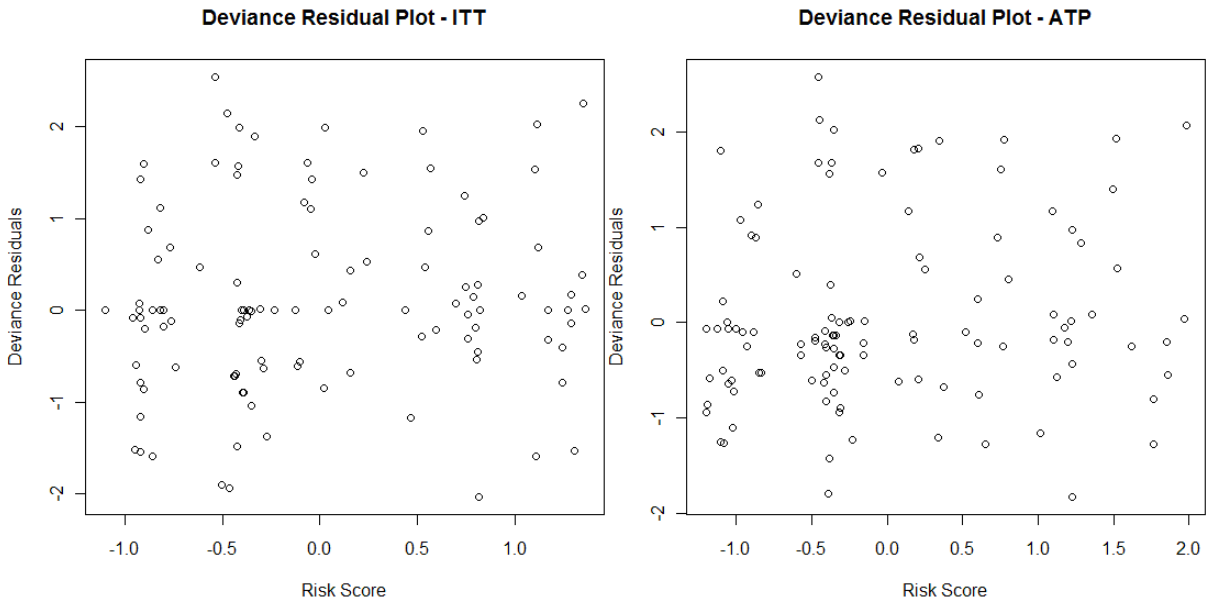


Figure 8: *Deviance residuals against the values of the risk score (ITT-left, ATP-right)*

5.3.3.2 According to Protocol Cohort

From Figure 8 (*right panel*), a scatter of random noise about zero was observed. These show that the individual fitted well the data, however, some observation appeared to be outlier.

A plot of observed and expected survival from Kaplan- Meir and Cox model, respectively, was employed to see, if any, the discrepancy between observed and predicted survival. Figure 9 (*right panel*) shows the two graphs are very close, which also suggested the fitted model was good.

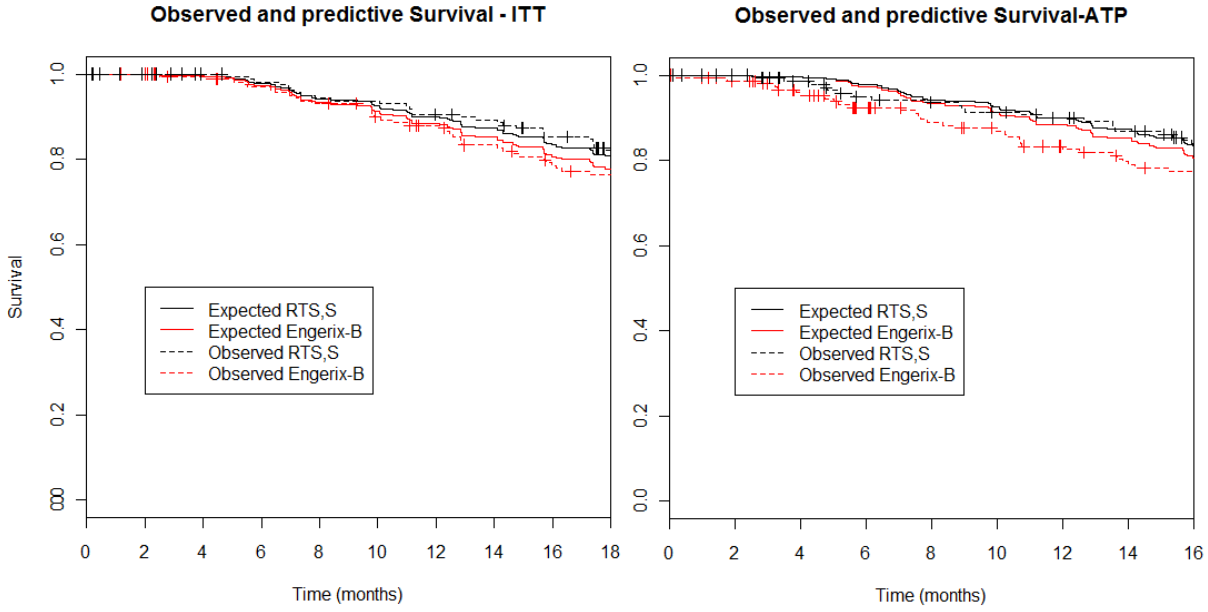


Figure 9: *Observed Versus Expected Plot for Survival per Treatment Group(ITT-left,ATP-right)*

5.3.4 Identification of Influential Observations

In section 5.3.3 we have seen some indication of outlying observations, therefore, this section was intended to assess the influence of the suspected individuals.

To study the influence of the suspected subject, the approximate standardised delta-beta was used. The subjects with largest or smallest values of deviance residuals were examined for influential.

5.3.4.1 Intention to Treat Cohort

Even though the deviance residuals plot (Figure 8, *left panel*) shows the subject number 268 to have the highest value of the residual, equal to 2.839, the results from delta-beta revealed that the subject number 304 had the largest delta-beta value, which was equal to 0.029. However, both subjects were investigated for influence on the estimate.

The actual change in parameter estimate of the treatment on omitting the data for subject 304 was 0.030. The standard error of the parameter estimate of the treatment in the ITT cohort was 0.235. Therefore, the change in the estimate on deleting subject 304 was 0.128 standard error. The omission of subject 268 caused the change in estimate of 0.008 and the maximum amount, by which this estimate change when deleting subject 268, was 0.034 standard error. This suggests that these observations were not influential.

5.3.4.2 According to Protocol Cohort

From Figure 8 (*right panel*), subject number 255 have the highest value of the deviance residual, which is equal to 2.812. On the other hand, subject number 304 appeares to have the largest delta-beta value, which is equal to 0.039. Both subjects were investigated for their influence on the model.

The actual change in parameter estimate of the treatment induced by omitting the data for subject 304 was 0.040. The standard error of the parameter estimate in the ATP cohort was 0.261. Therefore, the change in the estimate induced deleting subject 304 was equal to 0.153 standard error.

Similarly, omission of subject 255 caused the change in estimate of 0.040, which was equal to 0.153 the estimates' standard error. This suggests that these observations were not influential.

5.3.5 Time Dependent Covariate

5.3.5.1 Intention to Treat Cohort

From Figure 5, a linear function of survival time was suggested, therefore, a Cox model was then extended to incorporate a linear function of time and a test of zero slope was done.

The results from fitting a model with interaction between treatment effect and linear function of time, revealed no significant interaction effect (p-value=0.1349). This confirms our earlier observation that a constant-in-time treatment effect can be assumed.

5.3.5.2 According to Protocol Cohort

A linear function of survival time observed in Figure 6 was further tested to see if the coefficient for the time-dependent covariate is zero.

The results from fitting a model with interaction between treatment effect and linear function of time, revealed that the coefficient for the time-dependent covariate is zero (p-value=0.1877). This suggests that a constant-in-time treatment effect can be assumed.

6 Discussion and Conclusion

This report is the extension of the study reported by Abdulla *et al*, aims to describe the results of the vaccine efficacy against febrile malaria (as defined in Table 1) up to 17.5 months of follow-up.

The efficacy endpoint was time to the first febrile malaria infection observed over 17.5 months.

The Cox proportional hazard model was chosen to analysis the data. This methodology has the advantage of using all available information, including patients who fail to complete the study.

An attractive feature of Cox model is that Cox's semi-parametric modeling allows for no assumption to be made about the parametric distribution of survival times, making the method considerably more robust. Beside of this nice feature, Cox model really on strong assumption of proportionality, this must be checked in order for the results to be reliable.

In our analysis scaled Schoenfeld residuals have been implemented to visually check if there was a departure from the PH assumption. A smoothed plot of scaled Schoenfeld residual against rank of survival time showed no trend over time. A formal test was then performed by including the interaction between treatment effect and time. The Wald test showed no significant time effect. Thus, the model diagnostics didn't detect a violation of the proportional hazard assumption. This means that a model with no time varying treatment effect is appropriate.

However, in this study we focus only on testing proportional hazard assumption of treatment, it is also possible for proportional hazard assumption to be violated by baseline variables; distance and area. Therefore, thorough analysis investigating the proportional hazard assumption with respect to these other variables is recommended.

Because model-based inferences depend completely on the fitted statistical model, the adequacy of the model was assessed. Different residuals were applied to examine different aspects of the Cox model fitted.

The results from martingale residuals, suggested no need for any other forms for the distance covariate than the linear form. On the other hand, the plot of deviance residuals showed a satisfactory fit of the model to the data, with some possible outlying observation nevertheless. However, these outlying observations did not have any influence to the estimated parameters.

Based on the assessment of model adequacy we concluded that the overall fit of the model was good.

After completing all model diagnostics procedures fit, the results from fitting the Cox model, gave the hazard rate reduction of 33% over the 17.5 months of follow up period. The reduction was not statistically significant (p-value=0.1256).

This efficacy estimate is lower than the 43% reduction over 9 months follow-up reported by Abdulla *et at,* and also lower than the one against first malaria infection (defined as above) previously reported.[1,3] On the other hand the vaccine efficacy against febrile malaria in this study is lower than the 53% over 8 months follow-up reported previous.[5]

Infants living far from Bagamoyo District hospital (area 1 and 2) have higher risk of infection as compared to those infants living very close (area 5) to the District hospital, however this difference is not significant.

The risk of febrile malaria was seen to increase significantly by 0.082 with a one kilometer increase from heath centre; this might be the consequence of poor health seeking behavior for mother living far from hospital. Mother who lived far from hospital might have been tending to wait for long, and gave their baby herbs medication or sent them to a witchcraft, until their baby become seriously sick before sending them to the hospital for treatment.

The incidence of febrile malaria at the end of follow-up was higher in Engerix-B group than in RTS,S group (0.19 *vs* 0.14), but this difference was not statistically significant. This rate was slightly higher than 0.15 *vs* 0.10 respectively, reported in the same study, when the follow-up up to 9 months was considered. The low rate of detection of infection through active surveillance is likely to be a result of improved malaria control associated with distribution of bed nets, along with a close follow-up and improved clinical care of the infants in our study.[1]

Malaria vaccine has been shown to work well against first malaria infection with P. *falciparum*, for the first 9 months post dose one[1]. Given the limited sample size and number of new malaria case, the analysis did not have enough power to show the efficacy of the RTS,S against febrile malaria infection, over 17.5 months of follow-up period.

The analysis was performed in two cohorts: intention-to-treat and according-to-protocol. Our conclusion was based on according-to-protocol cohort; this is due to the fact that the immunity that the vaccine thought to provided takes times to develop to the body, unlike

the drugs which take immediate effect once after administration. Other reason, which makes us to base our conclusion on according-to-protocol cohort is that, based on the study design, which required that each infant have to receive the clearance drug for malaria parasitemia, and hence to be not infected prior the start of follow-up which is not generated in intention-to-treat cohort.

Because according-to-protocol analysis excludes subjects who are not fulfills the protocol requirements, the results may be subjected with bias. Therefore, the intention-to-treat results is more reliable than according-to-protocol analysis.

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