

Background

Malaria still kills almost 2,000 people each day, mainly children under the age of five. Artemisinin-based combination therapy is the first-line treatment against *falciparum* malaria [1]. However, artemisinin-resistant *falciparum* malaria is emerging in Southeast Asia, threatening our ability to control and eliminate malaria.

Methods

- 59 patients were recruited in southern Myanmar and received oral artesunate monotherapy (4 mg/kg/day) for 7 days [2].
- PK observations up to 8 hours post-dose, and parasite counts every 12 hours until 2 consecutive negative readings were obtained.
- Nonlinear mixed-effects modelling were used to characterize PK and PD properties using NONMEM 7.3.
- Individual LLOQs of parasite measurements were calculated, based on patient's weight and a minimum plausible observed parasitemia.
- An informative prior from a Thai-Cambodian boarder population was applied to support the estimation of PD parameters [3].

Objective

The aim of this work was to evaluate the PK/PD properties of artesunate (ARS) and its active metabolite, dihydroartemisinin (DHA), in drug-sensitive and drug-resistant *falciparum* malaria infections in order to quantify the impact of artemisinin resistance in Southern Myanmar.

Conclusions

- The PK/PD properties of ARS and DHA were well characterized and the implemented mixture model was able to differentiate between drug-sensitive and drug-resistant parasites in this population.
- The estimated resistant probability in Myanmar population was 56%.
- The collected data were also used as an external validation of a simple nomogram (previously developed to differentiate clinically between resistant and sensitive infections).
- The predictive value obtained here, justified the utilization of this nomogram in routine clinical trials.

Results and Discussion

Pharmacokinetics

- The PK model was best described using a transit compartment (n=3) absorption model and complete *in vivo* conversion of ARS into DHA (Fig 2). Both parent and metabolite were well described by one-compartment disposition kinetics (Fig. 1).

Pharmacodynamics

- The parasite-killing effect of ARS/DHA was described accurately using an effect compartment in order to produce a delayed Emax response (Fig. 2).
- A mixture model was implemented on Emax to quantify the difference between drug-sensitive (β_S) and drug-resistant (β_R) parasites (Eq. 1a & 1b).

$$Emax_{RESISTANT} = \beta_R * exp(\eta_R)$$
Eq. 1a

$$Emax_{SENSITIVE} = \beta_R + (\beta_S - \beta_R) * exp(\eta_S)$$
Eq. 1b

- The estimated Emax value was similar to the prior value (0.269) for sensitive parasites, but somewhat lower (18%) for resistant parasites when comparing it to the prior value.
- The estimated drug-dependent parasite clearance half-lives were 3.86 hr and 6.67 hr for sensitive and resistant parasite infections, respectively.
- The estimated EC₅₀-value (25 nM) was in agreement with that in the Thai-Cambodia population (30nM).
- The data presented here was used as an external validation of a previously developed nomogram (i.e. using a ratio of baseline to day1,day2,day3 parasitemia, respectively, to differentiate between resistant and sensitive malaria infections). The predictive performance showed 86.2% sensitivity and 86.8% specificity of the applied nomogram.

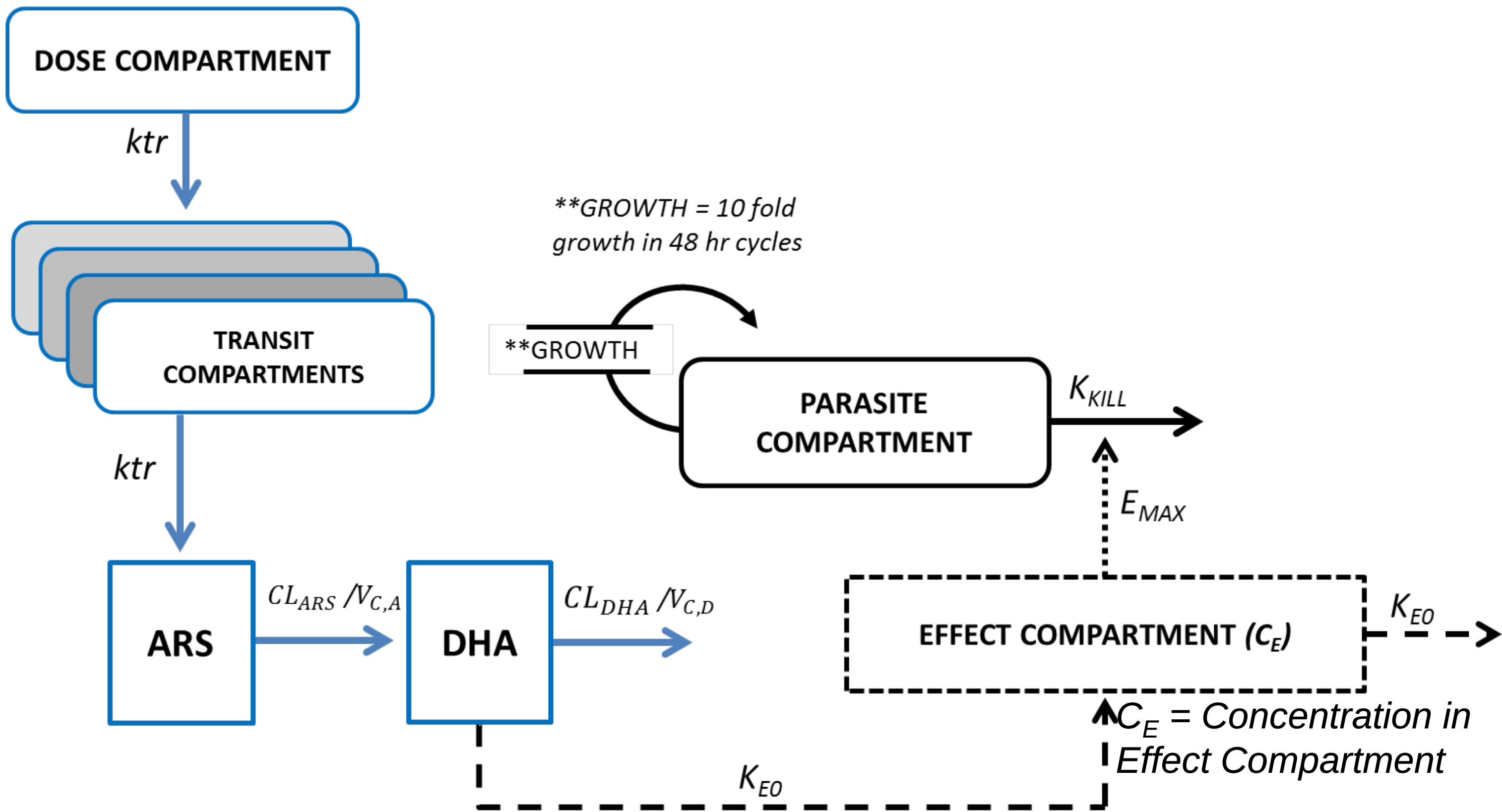


Fig. 2. Schematic structure of the final PKPD model.

Table 1. Final PK-PD model parameter estimates.

Parameter	Estimates (% RSE)	%CV BSV (% RSE)
Pharmacokinetics		
Artesunate (ARS)		
F	1 fix	31.9 (29.4)
MTT (h)	0.476 (8.6)	59.7 (24.9)
CL/F (L/h)	846 (6.9)	26.8 (47.7)
V _{ARS} (L)	649 (12.2)	69.5 (25.8)
RUV (add. Log-scale)	0.726 (3.82)	-
Dihydroartemisinin (DHA)		
CL/F (L/h)	37 (5.4)	19.5 (53.5)
V _{DHA} (L)	50 (6.7)	25.6 (62.7)
RUV (add. Log-scale)	0.582 (3.62)	-
Pharmacodynamics		
Parasite multiplication (/48 hr)	10 fix	-
Baseline parasitemia (Log ₁₀)	11 (0.7)	4.2 (23.1)
k _{e0} (h ⁻¹)	0.109 (39.1)	-
EC ₅₀ (nM)	25 (35.8)	-
Emax (sensitive)	0.259 (5.79)	35.7* (30.2)
Emax (resistant)	0.15 (5.37)	11.7 (81.6)
Prob. of mix2 (resistant)	0.56 (18.7)	-
RUV (add. Log-scale)	0.333 (5.95)	-

* SD was calculated from a simulated Emax distribution of 10,000 patients.

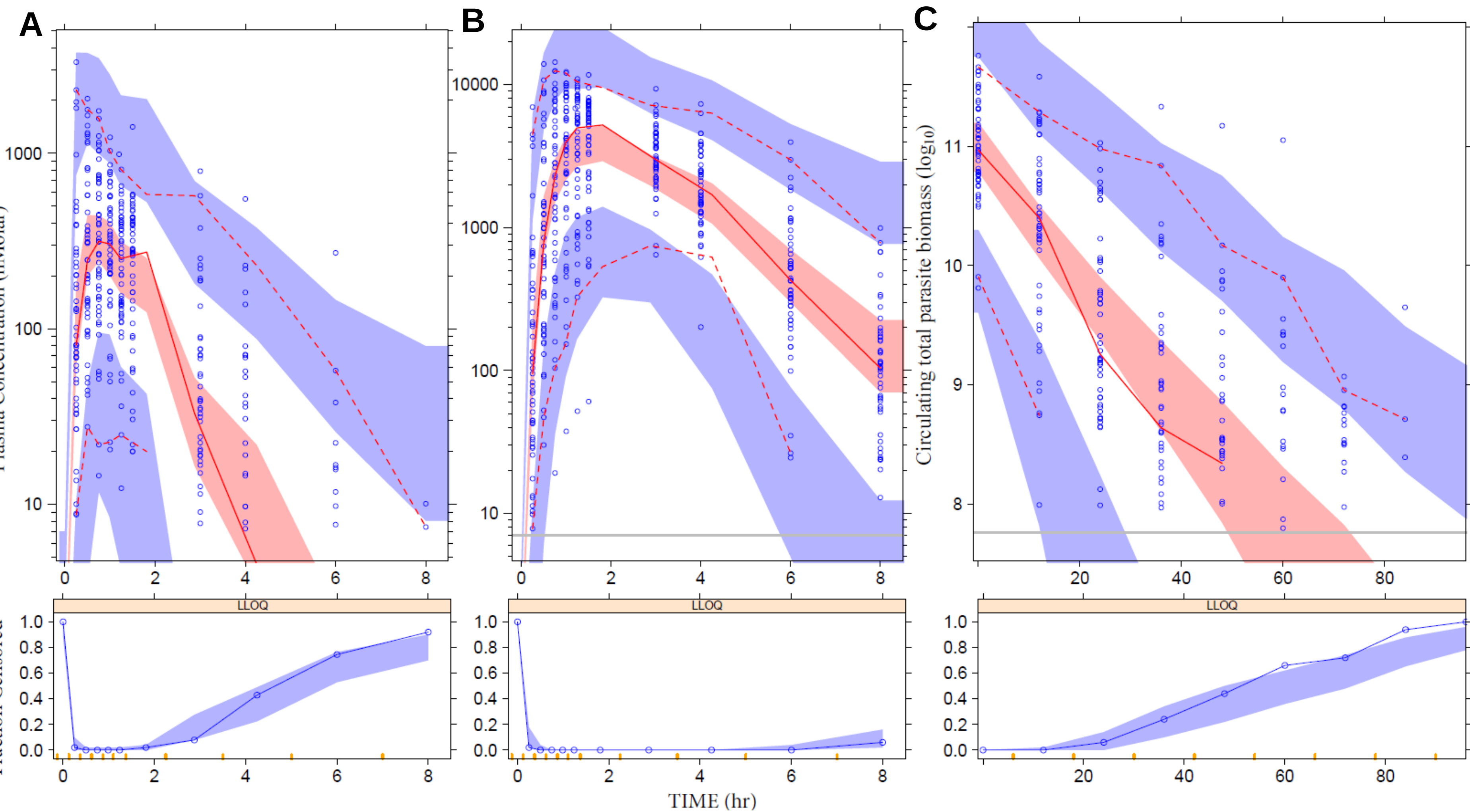


Fig. 1. Visual Predictive Check (VPC) of final population pharmacokinetic model: (A) Artesunate, (B) Dihydroartemisinin and (C) final pharmacodynamic model.

References

[1] WHO, World Malaria Report 2015. 2015.
[2] Kyaw, et al. Reduced Susceptibility of Plasmodium falciparum to Artesunate in Southern Myanmar. PLoS ONE, 2013. 8(3): p. e57689.
[3] Permala, et al. Population PK/PD Modelling of Emerging Artemisinin Resistance in Southeast Asia. ICAAC. 2015

- A mixture model was implemented on Emax to quantify the difference between drug-sensitive and drug-resistant parasites.

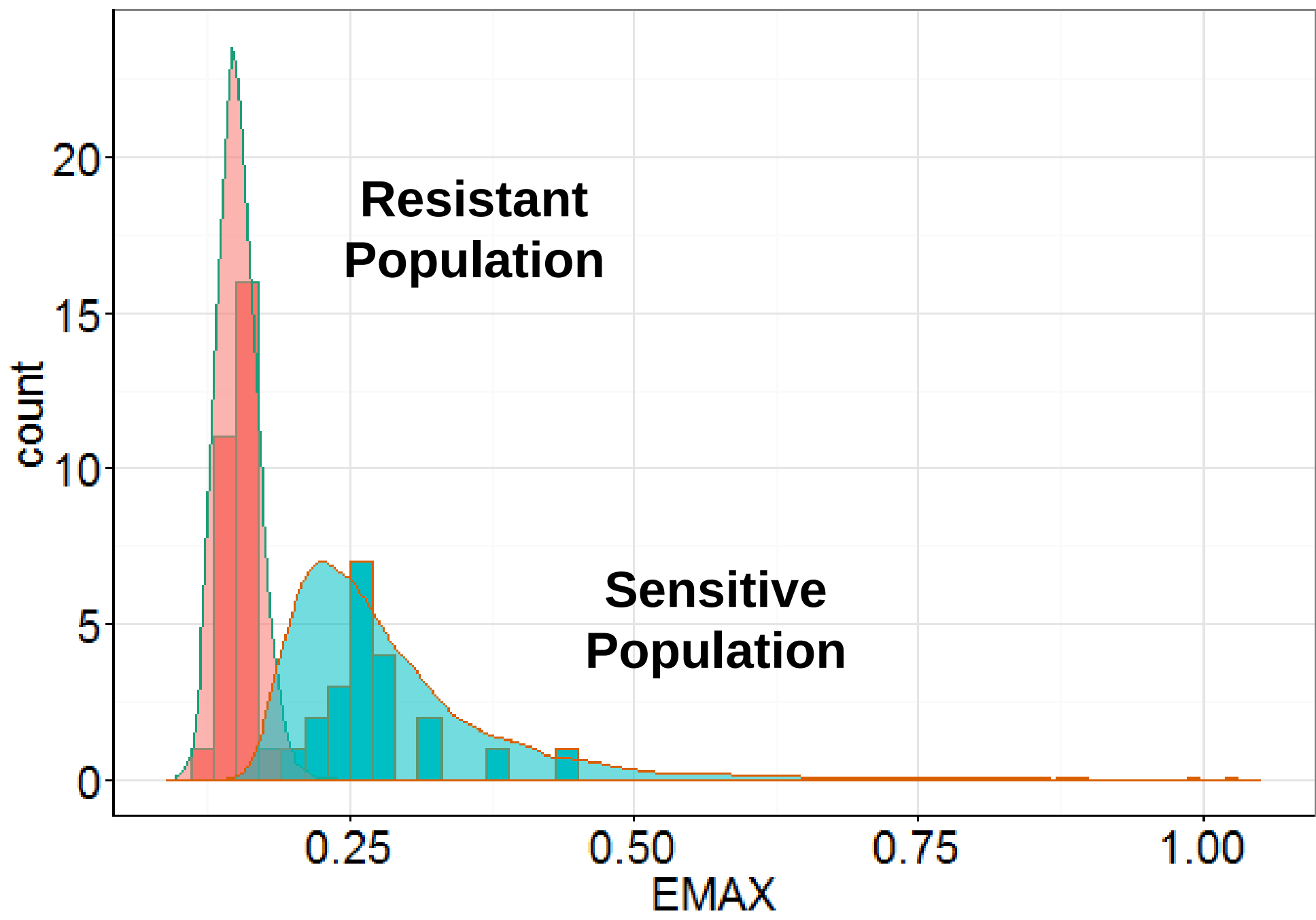


Fig. 3. Schematics of model estimated Emax distribution of the resistant and sensitive population.

Nomogram

- A simple nomogram which uses the ratio of baseline parasitemia to parasitemia at day1/day2/day3 to differentiate between resistant and sensitive malaria infection was developed.
- Baseline adaptive nomogram was able to predict resistant infection with > 75% True Positivity rate (Fig.4)

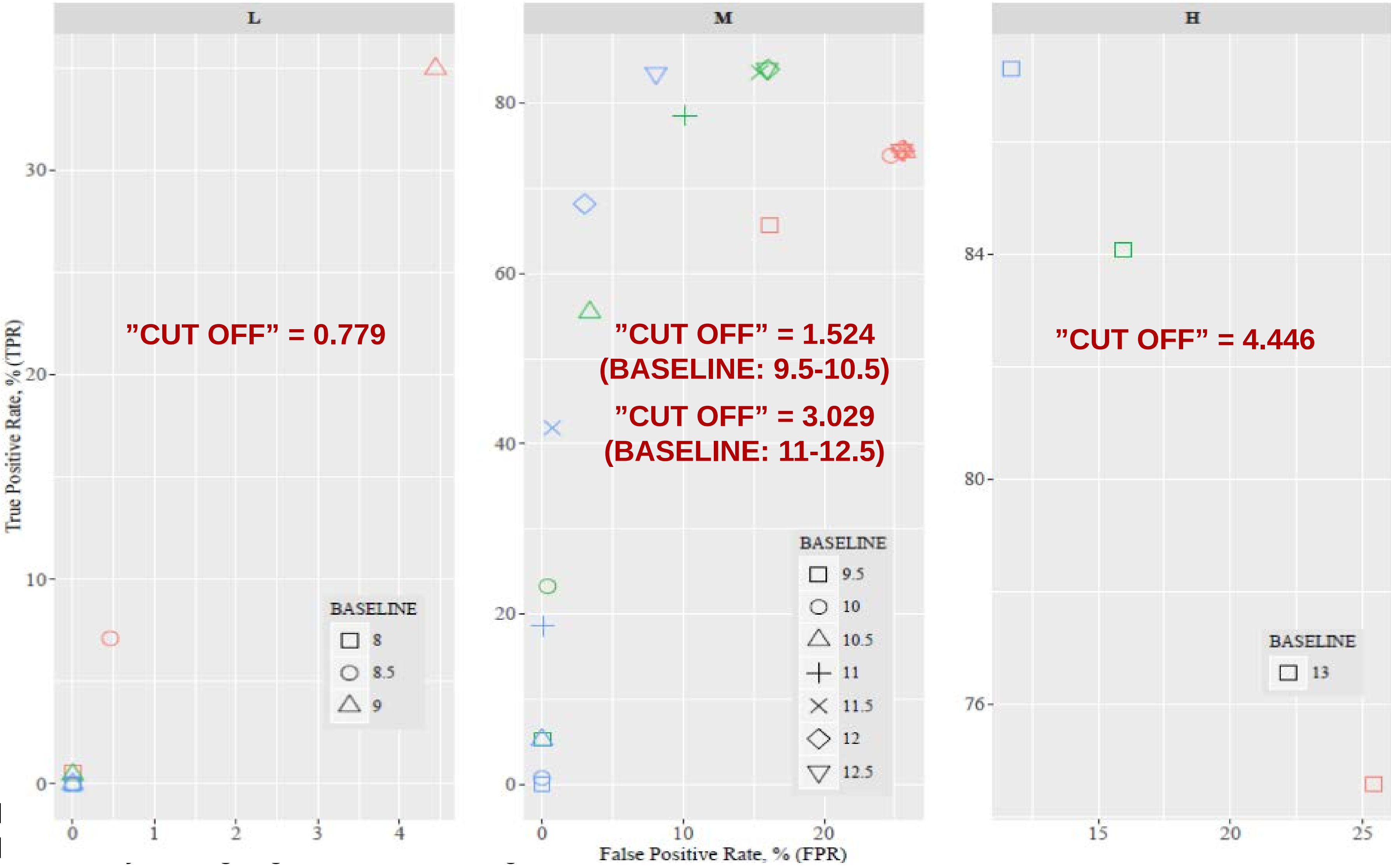


Fig. 4. The nomogram performance, True Positive Rate (%), in large simulated population (n=10,000) at day1 (red shape), day2 (green shape) and day3 (blue shape) respective to baseline parasite density (baseline density of log₁₀ parasitemia ranging from 8 – 13) (L=low density, M=moderate density, H=high density)

- For baseline density of :
 - 8-9, the nomogram deem not suitable due to low precision
 - 9.5 -10.5, the distinction can is optimal at Day-1 (“CUT OFF” , value =1.524)
 - 11-12.5, the distinction is optimal at Day-2 (“CUT OFF” value = 3.029)
 - 13, the distinction is optimal at Day-3 (“CUT OFF” value = 4.446

- The developed nomogram was implemented on the Myanmar population data as described in the steps below (Fig 5) to serve as external validation

The ratio of baseline parasitemia to parasitemia at day1, day2, day3 was obtained.

The ratio was compared to the respective “CUT OFF” value depending on the baseline density

Ratio above the “CUT OFF” value was classified as sensitive while value below ” as resistant.

The classifications were compared to patient’s probability assigned by the model and sensitivity analysis was performed

Fig. 5. Schematics of the proses where nomogram was applied on Myanmar data as external validation

Table 2. Final PK-PD model parameter estimates of Thai- Cambodia population

Parameter	Estimates (% RSE)	%CV BSV (% RSE)
Pharmacokinetics		
Artesunate (ARS)		
F	1 fix	32.6 (27.4)
MTT (h)	1.34 (6.4)	59.7 (24.9)
CL/F (L/h)	1060 (5.2)	-
V _{ARS} (L)	307 (9.2)	74 (19.4)
RUV (add. Log-scale)	1.13 (3.04)	-
Dihydroartemisinin (DHA)		
CL/F (L/h)	137 (4.4)	5.5 (27)
V _{DHA} (L)	151 (4.9)	21.4 (18.07)
RUV (add. Log-scale)	0.6 (2.3)	-
Pharmacodynamics		
Parasite multiplication (/48 hr)	10 fix	-
Baseline parasitemia (Log ₁₀)		
Pailin, Cambodia	11.4 (0.79)	5.2(18.07)
Wang Pa, Thailand	11 (0.97)	6.3 (24.26)
k _{e0} (h ⁻¹)	0.0761 (15.75)	-
EC ₅₀ (nM)	30 (32.5)	-
Emax (sensitive)	0.269 (5.79)	47.5* (38.3)
Emax (resistant)	0.183 (5.37)	10.3 (36.29)
Prob. of mix2 (resistant)		
Pailin, Cambodia	0.838(9.33)	-
Wang Pa, Thailand	0.165 (51.6)	-
RUV (add. Log-scale)	0.352 (2.47)	-

* SD was calculated from a simulated Emax distribution of 10,000 patients.

Table 3. The baseline adaptive nomogram performance on Myanmar population data, in comparison to non baseline adaptive “CUT OFF” and day-3 positivity test

Statistics Metric	Interpretation	Value (%)		
		Baseline Adaptive	Non Baseline Adaptive	DAY-3 POSITIVITY
Negative Result	Non-resistant if the ratio value is above the "CUT OFF" value			Non-resistant if parasitemia reading are below LLOQ* at day 3 post dose
Positive Result	Resistant if the ratio value is below the "CUT OFF" value			Resistant if parasitemia reading are above LLOQ* at day 3 post dose
True Positive	The nomogram correctly identifies resistant	86.2	86.2	44.8
False Positive	The nomogram in-correctly identifies resistant	13.8	13.7	55.2
True Negative	The nomogram correctly identifies sensitive	90.5	90.5	100
False Negative	The nomogram in-correctly identifies sensitive	9.5	9.5	0
Sensitivity	Ability to correctly resistant	86.20	86.29	44.80
Accuracy		88.35	88.39	72.4

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