Optimal treatments for severe malaria and the threat posed by artemisinin resistance

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Footnotes

Conflict of interest statement

The authors do not have a commercial or other association that might pose a conflict of interest.

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Abstract

Background:

Standard treatment for severe malaria is with artesunate; patient survival in the 24 hours immediately post-treatment is the key objective. Clinical trials use clearance rates of circulating parasites as their clinical outcome, but the pathology of severe malaria is attributed primarily to non-circulating, sequestered, parasites, so there is a disconnect between existing clinical metrics and objectives.

Methods & Findings:

We extend existing PK/PD modelling methods to simulate the treatment of 10,000 patients with severe malaria and track the pathology caused by sequestered parasites.

Our model recovered the clinical outcomes of existing studies (based on circulating parasites) and showed a "simplified" artesunate regimen was non-inferior to the existing WHO regimen across the patient population but resulted in worse outcomes in a sub-group of patients with infections clustered in early stages of the parasite life-cycle. This same group of patients were extremely vulnerable to resistance emerging in parasite early ring stages.

Conclusions:

We quantify patient outcomes in a manner appropriate for severe malaria with a flexible framework that allows future researchers to implement their beliefs about underlying pathology. We highlight with some urgency the threat posed to treatment of severe malaria by artemisinin resistance in parasite early ring-stages.

Key words

malaria, falciparum; malaria; artesunate; artemisinin; computer simulation; pharmacology; clinical; sequestration; pharmacokinetics;

Background

Plasmodium falciparum is the malaria species responsible for the largest number of deaths worldwide[1] and presents clinically in two forms. Patients with "uncomplicated" malaria have a relatively mild fever, are conscious and capable of taking oral drug regimens; prompt treatment of uncomplicated malaria is associated with low mortality [2]. Patients with "severe" malaria present with one, or a combination, of four syndromes: Severe anaemia, respiratory distress, metabolic derangement and cerebral malaria [3, 4]. Patients are treated with parenteral artesunate, which rapidly kills parasites, but resolution of pathology lags behind parasite killing; case fatality rates are high even once patients have been admitted to the formal health system (typically between 5 and 12% [2] although these have been falling to ~2% [5]).

A key factor responsible for severe malaria is the binding of parasitized erythrocytes (subsequently called infected red blood cells, iRBCs) to microvascular endothelium, a process known as sequestration. iRBC sequestration induces pathology through three main causes: (i) impairing blood flow to organs through direct physical blockage of the capillaries [6], (ii) indirect blockage via host defence mechanisms such as inflammation [7] and (iii) physical damage to microvascular endothelium and the blood/brain barrier [8]. High case fatality rates occur, even if the drug kills parasites within sequestered iRBCs, because the molecules responsible for sequestration (for example, *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) [9]) are still present on iRBC surfaces and it takes a

significant amount of time for these ligands to decline sufficiently for the sequestered iRBC to detach and/or for the pathology associated with sequestration to resolve [10, 11].

Parasite clearance rates are a commonly used clinical outcome measure to compare efficacy of antimalarial treatment regimens. However, parasite clearance rates correlate poorly with disease outcome in severe malaria. Large trials comparing intramuscular artemether with quinine in African children showed more rapid parasite clearance with artemether but no difference in case fatality [12, 13]. With parenteral artesunate, parasite clearance rates are not different in patients dying from severe malaria compared to survivors (results cited in [14]). There are two potential explanations why parasite clearance is an unsuitable outcome measure in severe malaria: Firstly, parasite clearance rates following treatment for uncomplicated malaria appear to mainly reflect host immunity rather than drug effectiveness [15-17] so may be a poor metric of overall drug effectiveness. Secondly, parasite clearance rates are measured on circulating parasites [15] whereas non-circulating, sequestered parasites are responsible for most clinical symptoms, pathology and deaths associated with severe malaria [3]. We developed a new model based on existing pharmacokinetic/pharmacodynamic (PK/PD) models [18, 19] (themselves based on [20-22]) to investigate two simple metrics reflecting the pathology of sequestered parasites in severe malaria: The maximum sequestered load post-treatment, and the area under the curve (AUC) of sequestered parasites over time post-treatment. We quantified and compared the impact of existing and proposed drug regimens on these metrics to identify rational drug dosing regimens for treatment of severe malaria. Additionally, we quantified the likely impact of artemisinin resistance in treatment of severe malaria.

Methods

We utilized a computer-based PK/PD model to track changes in the number of sequestered iRBCs following drug administration. The model was implemented in the statistical programming software R [23] version 3.4.1. *P. falciparum* parasites undergo a 48-hour developmental cycle in human erythrocytes with two main implications for pathology and treatment. Firstly, parasites initially circulate freely in blood vessels but sequester (i.e. bind to capillaries) at mature stages of their intra-erythrocytic cycle. Secondly, parasites differ in their sensitivity to drugs over the course of this 48-hour cycle.

As previously described [22], we separated the parasite population within a patient into 48 'age-bins' that each represent a one-hour long development stage in the parasite's 48-hour life-cycle within human erythrocytes. Parasites within age-bins have differing propensities to sequester and have varying degrees of drug sensitivity. Our model tracked the number of iRBCs in each of four classes at any time post-treatment depending on whether the parasites are alive or dead, and whether the iRBC is circulating or sequestered: Alive & circulating, alive & sequestered, dead & circulating, and dead & sequestered (see Figure 1 for illustration). Note that iRBCs classed as "dead & sequestered" are those iRBCs whose parasites have died while sequestered and are either: (i) still sequestered and causing pathology or (ii) have ruptured/detached from the capillary but are still associated with continued, lingering pathology. For model specification and details, see Supplementary information.

Pathological load and pathological recovery rate

Severity of the malaria infection is determined by what we refer to as 'pathological load', i.e. the number of sequestered iRBCs (containing either living or dead parasites) physically restricting blood flow and/or eliciting patient's immune and/or inflammatory response that may also contribute to pathology [3, 24]. It is unlikely that the iRBC immediately ruptures on death of the parasites (which would reduce physical blockage of the capillary) or that the immune/inflammatory responses immediately disappear when the parasite dies, so we assumed that pathology persists for a period

after the death of the sequestered parasites. We captured this effect by defining a 'pathological recovery rate', *r*, which is the rate at which the pathology caused by sequestered iRBCs disappears with time following the death of the parasite. As will be discussed later, there are no clinical estimates of this 'recovery rate' so our strategy was to quantify the impact of dosing regimen and artemisinin resistance across a range of values of recovery rate to test whether our results were dependent on assumed values for recovery rate (we show later that they were not). We varied the 'recovery rate' *r* in the simulations by altering its half-life (Table 1), which is the time it takes pathology caused by dead sequestered parasites to reduce by half. We assumed that parasite death, with consequent rupturing of the iRBC or reduction of binding ligands (allowing iRBCs to detach from blood vessel walls), was essential to allow the start of pathological recovery, hence sequestered iRBCs with living parasites were not subject to the pathological recovery rate. We quantified the pathological load $L(t)$ at any time t post-treatment as the sum of the current number of sequestered iRBCs with living parasites $\alpha(t)$ and the lingering pathological effects of once-sequestered iRBC whose parasites were killed in the current or previous time periods, $\beta(i)$, i.e.

$$L(t) = \alpha(t) + \sum_{i=1}^{t} \beta(i) e^{-(t-i)r} \qquad \text{Equation 1}$$

We used two metrics to analyse treatment regimens and resistance: (i) Maximum pathological load (MPL), the maximum value of *L(t)* occurring during a defined time period post-treatment, and (ii) the area under the pathological load curve (AUC_{PL}) during a defined time period post-treatment, i.e. the total pathology in that period. For example, the AUC_{PL} in the period 0 to 24 hours post-treatment is:

$$AUC_{PL} = \sum_{t=1}^{24} L(t) \qquad \text{Equation 2}$$

<u>Simulating patient treatment cohorts</u>

We simulated a cohort of 10,000 patients who had parasitological, pharmacological, and patient-specific parameters drawn from the distributions given in Table 1. Individual patient profiles allowed individual PK/PD variation to be incorporated to generate individual patient post-treatment parasite clearance dynamics (Supplementary information). Each patient was simulated three times under different scenarios: Once for drug sensitive parasites treated by the standard WHO regimen (2.4mg/kg artesunate twice a day in the first 24h), once for sensitive parasites treated with the simplified regimen (4mg/kg artesunate once a day, as proposed by Kremsner *et. al* [25]), and once for artemisinin resistant parasites treated by the standard WHO regimen. This allowed us to compare the two dosing regimens ("standard" versus "simplified") and the impact of resistance ("sensitive" versus "resistant"), *in each patient*. Follow-up time was 48 hours after drug administration; this reflected a whole parasite life-cycle within an iRBC but, more importantly, covers the period post-treatment where a patient is most likely to die [26, 27].

Sensitivity analysis

We conducted partial rank correlation coefficient (PRCC) using Spearman's Rho to establish the strength of the relationship between model parameters and dependent variables (i.e. the pathology metrics AUC_{PL} and MPL).

All parameters are quantitative so can enter the PRCC without modification. The exception is mean age-bin which, although numeric, has a 'circular' scale, age-bin 1 being adjacent to age-bin 48, due to parasites from ruptured iRBCs (at hour 48) reinvading to restart the asexual lifecycle. The mean age-bin variable was therefore split into either 5 or 3 ordinal classes (depending on whether parasites were hyper-sensitive or resistant to artemisinin) as described in Supplementary information.

The following parameters were included in the PRCC analysis:

- Duration of artesunate killing post-treatment; this captures all the PK/PD parameters in Table 1 except maximal artesunate kill rate
- Maximal rate of artesunate killing (V_{max})
- Initial mean age-bin as a categorical variable (see above)
- Variation of initial age-bin distribution (measured as the standard deviation (SD) around the mean).
- Initial parasite number
- Parasite multiplication rate (PMR)
- Half-life of the 'pathological recovery rate' (r)

The splenic clearance rate was not included in the analysis as it has no impact on sequestered iRBC based pathology.

Results

Our model calculated pathological load and returns two outcome metrics: AUC_{PL} and MPL. **Figure 3** shows the values of these metrics for 3 model scenarios: Patients with sensitive parasites treated with the standard WHO regimen, a comparison of the ratios of AUC_{PL} and MPL for treatment with simplified regimen v standard regimen, and the impact or artemisinin resistance on outcomes following treatment with standard WHO regimen.

Ratios of outcome metrics are calculated as simplified regimens scaled by standard regimen and as resistant parasites scaled by sensitive parasites. High metrics are deleterious, thus ratios of >1 indicate worse prognosis associated with the simplified or resistant parasites. These ratios quantify the impact e.g. a ratio of 5 for resistant vs sensitive parasites indicates pathological metrics are 5 times higher when treating resistant parasites. We investigated four time periods post-treatment: 0-12h, 0-24h,

12-24h and 24-48h.

Consistency of model outputs with existing field data

Our model calculated parasite reduction ratios (PRR) from circulating parasite numbers (Supplementary information). The clinical endpoint of the trials by Kremsner and colleagues was the proportion of patients in each arm whose PRR at 24 hours (PRR_{24}) was >99% [25], reported as 79% and 78% for the five-dose standard and the three-dose simplified regimen, respectively. When calibrated with PK parameters from Kremsner's study [25], our results were consistent with these clinical observations, i.e. our model predicted 78% and 74% for the standard and simplified regimen with hyper-sensitive parasites, respectively (S3 Table). However, the results we present below are calibrated using PK parameters from Hendriksen *et al.* [28] = (see Supplementary information for justification), with which we observed lower values of 70% and 62% of patients with PRR_{24}>99% for the standard and simplified i.m regimens, respectively.

Hendriksen *et al.* [28] do not report the percentage of patients with PRR_{24} > 99% in their study, so we could not simultaneously compare the findings of our simulation with the findings of Kremsner *et al*. [25] and Hendriksen *et al.* [28]. However, Hendriksen *et al.* [28] reported the population geometric mean of the fractional reduction in parasite counts at 24 hours as 96% (94-98%, 95% CI) following treatment with the standard regimen. The population geometric mean obtained for the reduction in parasite counts at 24 hours (i.e. PRR_{24}) in our simulation using parameters from Hendriksen *et al.* [28] was >99%.

The general accepted value for PRR_{48} following artemisinin treatment is 10^{-4} [29] which is very close to the value obtained here: For the standard regimen, using the artesunate duration derived from Hendriksen's PK parameters (Figure 2) we obtained a mean PRR_{48} of 5.18^{-5} (Supplementary information for a nuanced discussion of PK parameters).

220

221 Standard regimen treatment of artemisinin-sensitive parasites

222 We simulated treatment of drug-sensitive parasites with the standard regimen and identified the key
223 drivers of pathology by calculating which parameters were most correlated with AUC_{PL} and MPL
224 (Figure 4; S7 Table). The most highly correlated parameter for both metrics was the initial parasite
225 number: Large positive PRCCs (between 0.88 and 0.98) were observed with associated *p* values
226 <=0.001 at all time-periods. The half-life of the recovery rate *r* had PRCC of 0.46 for AUC_{PL} and 0.34 for
227 MPL in the 24-48h time-period (*p* values <=0.001), but PRCC of <0.3 in earlier time periods. All other
228 parameters had PRCC values of <0.3, indicating that outcome metrics were not highly correlated as
229 per accepted statistical criteria [30]. All other model parameters had negligible correlation. The most
230 likely explanation is that such a large proportion of parasites are killed by artesunate that small
231 differences in number killed are negligible compared to the initial parasite number and pathological
232 recovery rate.

233

234 Comparison of simplified and standard regimen

235 We evaluated alternative treatment regimens on artemisinin-sensitive parasites. These results are
236 presented as ratios of AUC_{PL} and MPL. The simplified regimen had a slightly higher median ratio in 0-
237 24h of 1.03; MPL was 1. At 24-48h, higher medians of 1.49 and 1.45 for AUC_{PL} and MPL respectively
238 were observed (Figure 3; S4 Table).

239 Parameter analysis with PRCC (S8 table) revealed that patients whose initial infections were in either
240 very late or very early initial mean age-bins (Figure 5, lower panel) will have worse outcomes with the
241 simplified regimen. This occurred because parasites in these stages are largely insensitive to
242 artesunate at first treatment, and the simplified regimen lacks the second dose, 12 hours later, of the

243 standard regimen that would effectively target these parasites that had matured into more
244 artemisinin sensitive age-bins.

245 The half-life of the recovery rate *r* had a moderate correlation with outputs in the 12-24h and 24-48h
246 periods indicating that assumption of slower recovery made the simplified regimen perform relatively
247 better (S5 Figure). We are confident this parameter does not affect the validity of our results; for
248 complete discussion see Supplementary information. No other parameters have notable correlation
249 with sequestration-based pathology when comparing regimens. This is probably because they "cancel
250 out" as explained above e.g. initial parasite numbers is the same within patients thus cancels when
251 comparing the impact of different regimens within the same patient

252 We repeated this analysis to compare regimens when treating resistant (as opposed to drug-sensitive)
253 parasites. Results were extremely similar to those shown in Figure 5 (S7 Figure; S9 Table).

254

255 <u>The impact of artemisinin resistance on treatment by the standard regimen.</u>

256 Unsurprisingly ratios of AUC_{PL} and MPL when comparing resistant and sensitive parasites are never
257 less than 1 (Figure 3) i.e. under no circumstance did patients have a better outcome when parasites
258 are resistant. Differences in median values (Figure 3; S4 Table) were extremely small.

259

260 We carried out PRRC analysis (S10 Table) to investigate whether this small difference obscured the
261 presence of a vulnerable sub-group of patients. This appeared to be the case: Patients whose
262 infections are clustered in the early age-bins at time of treatment had pathological outcomes which
263 were significantly worse in the presence of resistance (Figure 6).

264 In these early age bins, ratios for AUC_{PL} and MPL are as high as 5 in the 0-24h period (comparisons
265 based on the upper quartile value). This occurs because artesunate presence post-treatment largely

coincides with parasites in age-bins insensitive to artesunate through resistance, rendering the initial dose nearly or completely ineffective.

SD of the initial mean age-bin had a positive correlation with the ratio (indicating that resistant parasites had worse outcomes as SD increased). This occurred because higher SD "nudged" parts of the age-bin distribution into (or out of) resistant age-bins (i.e. the contiguous bin 45-48 and 1-5 where killing is absent). PRCC analysis showed no other parameter had a PRCC value of >0.01, suggesting the initial mean age-bin (and, to a lesser extent, it's SD) are the sole determinants of whether a patient's outcome will be worse in the presence of resistance.

Discussion

We established a PK/PD modelling methodology capable of investigating the treatment of severe malaria. Kremsner *et al.* [31] recognised the clinical necessity of this, and noted that "for the first time, we [i.e. Kremsner *et al*.] are assessing artesunate using similar pharmacokinetic and dynamic approaches". Parasite clearance is likely to be a poor measure of regimen effectiveness (and, by extension, clinical outcome) in severe malaria where pathology is due to sequestered parasites. The effects of alternative regimens and the impact of drug resistance can only be investigated by traditional clinical outcomes using large scale clinical trials, so pharmacological modelling of the type proposed here is essential to help generate the evidence base for rational treatment design. Our pathological modelling was highly flexible (discussed in Supplementary information) and, of necessity, reflected the limitations in our understanding of pathology, for example, how rapidly pathology is resolved following parasite death and whether pathology depends on maximal sequestered load (measured as MPL) or on total exposure (measured as AUC_{PL}). An interesting, highly important result is that the key quantitative assumption made in the analysis, the rate of resolution of pathology

(measured as the half-life of *r*), had little effect on our conclusions when comparing alternative regimens or the impact of resistance (Supplementary information) implying that the pathological model is a robust to assumptions made in this comparative investigation Importantly, while circulating parasite loads do not reflect the pathology of severe malaria they are currently the regular endpoint of choice in severe malaria trials, including those undertaken by Kremsner *et al.* [25, 32]; our model was able to reproduce the clinical outcomes reported in [25, 28] (when appropriately parameterized), and recover expected PRR_{48}, so we are confident it is reflective of *in vivo* scenarios (Supplementary information).

Kremsner and colleagues [25, 32] concluded that their simplified regimen was non-inferior to the standard WHO regimen and possessed operational advantages due to less frequent drug administration[25, 32]. This work was influential and initiated a wider debate about the best drug regimen(s) to treat severe malaria [14, 31, 33] to which our study can contribute. Comparison of the 0-24h and 12-24h period was used to compare the effects of the initial, larger dose of the simplified regimen against the additional dose at 12h with the standard regimen. The standard regimen produced slightly lower median AUC_{PL} within the first 24 hours post-treatment (Figure 3; S4 Table). This difference was greater in the 24-48h period, but the majority of pathological load occurred within the first 24 hours as artesunate rapidly kills parasites– AUC_{PL} in the 24-48h period is, on average, between 20-30% that of AUC_{PL} in the 0-24h period (data not shown). The first 24 hours are critical for patient survival[26], so outcome metrics at 24-48h may have little relevance in choosing between regimens. However, the simplified regimen performed much worse in the sub-group of patients with very late or very early initial mean age-bins. Based on these results, we are dubious about recommending use of the simplified regimen but add an important rider to this. Kremsner *et al.* never claimed this simplified regimen would be superior, but argued that any inferiority, if it exists, would be within acceptable margins. We leave it to clinically qualified personnel to judge whether 50% in

some subgroups is within an acceptable margin of inferiority, especially given our inability to directly link our pathological outcomes with the likelihood of mortality.

We assessed the impact of artemisinin resistance on treatment of severe malaria, i.e. the extent to which resistance increased MPL and AUC_{PL}. Resistance prevents drug killing in age-bins 2-4 (these bins are otherwise hyper-sensitive) resulting in no killing fora contiguous 8 hour period in resistant parasites (i.e. age-bins 45 to 5). Our results show the initial mean age-bin and it's SD are the only parameters that distinguish outcomes between sensitive and resistance parasites (Figure 6) . We argued previously [34] that artemisinin resistance would have a negligible impact on eventual cure rates in uncomplicated malaria (provided there was no resistance to partner drugs) but artemisinin resistance clearly poses a much larger threat to treatment of severe malaria than it does to uncomplicated malaria. Although differences between sensitive and resistant parasites across the entire population are minor (Figure 3; S4 Table), there is an extremely vulnerable sub-group of patients whose infections at the time of treatment are clustered in very late or very early age-bins (i.e., where parasites are resistant in our model; Figure 6).

We present a highly adaptable methodology for PK/PD modelling of treatment of severe malaria that was able to recover key clinical observations (based on circulating parasite numbers), and, with novel metrics, used to investigate the pathology of severe malaria. Our model showed that while on a population level a simplified artesunate regimen is non-inferior to the standard WHO regimen, outcomes in a sub-group of patients with infections grouped in late or early initial mean age-bins are notably worse with the simplified regimen. The emergence of artemisinin resistance in early ring stages poses a significant threat to this same group of patients. Neither of these results are particularly obvious from summary statistics of the population and so sub-group analysis is particularly important in devising treatment strategies for severe malaria.

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