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A One-compartment Constant Rate Intravascular Infusion Model for the Evaluation of Increases in Hematocrit after Artemisinin-based Combination Treatments of Acute Falciparum Malaria in Children

Akintunde Sowunmi[1,2], Kazeem Akano[1], Adejumoke I. Ayede[3], Godwin Ntadom[4], Temitope Aderoyeje[5], Elsie O. Adewoye[6] and Bayo Fatunmbi[7]

[1]*Department of Pharmacology & Therapeutics, University of Ibadan, Ibadan, Nigeria*
[2]*Institute for Medical Research & Training, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[3]*Department of Paediatrics, University of Ibadan, Ibadan, Nigeria*
[4]*National Malaria Elimination Programme, Federal Ministry of Health, Abuja, Nigeria*
[5]*Department of Clinical Pharmacology, University College Hospital, Ibadan, Nigeria*
[6]*Department of Physiology, University of Ibadan, Ibadan, Nigeria*
[7]*World Health Organization, Regional Office for the Western Pacific, Cambodia*

Abstract

Increases in hematocrit frequently follow successful treatment of uncomplicated *Plasmodium falciparum* infections in children, but there is no pharmacokinetic model for the analyses of the increases in hematocrit following artemisinin-based combination treatments (ACTs) in malarious children. A one-compartment constant rate intravascular infusion model (CRIVIM), which employed the principles of constant rate intravenous infusion of drugs (CRIVID), was used to evaluate the kinetics of the increases in hematocrit after artesunate-amodiaquine (AA) or artemether-lumefantrine (AL) treatments in 112 malarious children. The model assumed baseline hematocrit was zero, a constant rate increase in hematocrit from baseline following treatment, and it involved semi-logarithm plots of the difference between hematocrit at plateau and that at earlier times, against the corresponding times. Hematocrit reached a plateau in a median time of 28 days after treatment started. Mean plateau hematocrit was 6.7% (95%CI 5.9.-7.5) and was similar in AA- and AL- treated children [6.8% (95%CI 6-7.7), *n* = 81 *v* 6.3% (95%CI 4.9-7.7), *n* = 31, *P* = 0.56]. Times to plateau were significantly shorter and plateau hematocrit significantly lower in non-anemic compared to anemic children. Overall, declines from semi-logarithm plots were monoexponential with mean half-time of hematocrit of 2.5 days (95%CI 2.2-2.8). Half-times were similar in AA and AL-treated children [2.4 days (95%CI 2.1-2.8) *v* 2.7 days (95%CI 2-3.3), *P* = 0.46], and were significantly shorter in anemic compared to non-anemic children [2.1 days (95%CI 1.8-2.4, *n* = 57) *v* 2.9 days (95%CI 2.4-3.5, *n* = 55), *P* = 0.01). Mean anemia recovery time was 13.8 days (95%CI 11.9 – 15.7). Bland-Altman analysis of 7 or 8 multiples of anaemia half-time and anaemia recovery times showed narrow limit of agreement with insignificant biases (P = 0.17 or 0.68, respectively). Steady state parameters were independent of baseline parasitemias. The one-compartment CRIVIM permits evaluation of increases in hematocrit following ACTs and may be used in observational and clinical studies in uncomplicated falciparum malaria.

Keywords: Malaria; Hematocrit; Constant rate infusion; ACTs; Children

Introduction

Acute *Plasmodium falciparum* infections in the non- and relatively non-immune are associated with variable declines in hematocrit which may fall below 30%, the lower threshold of normal [1,2]. In endemic areas of Africa, *P. falciparum* malaria-associated anemia (PfMAA), which has been attributed to destruction of parasitized and non-parasitized red blood cells, and bone marrow dyserythropoiesis of variable intensity and duration [1,3-9], is common in children [2,10-13].

Following successful treatment of acute falciparum infections, and in the absence of other underlying disorders, there are increases in hematocrit in virtually all non-immune and to a lesser extent, in a variable proportion of the relatively non-immune malarious Nigerian children [2,14]. The increases reach plateau or steady state level between 21 and 35 days after commencing treatment [2,14,15]. However, the rate of increase in hematocrit varies considerably between individuals [14]. The analyses of the increases following successful treatment of infections have been mainly descriptive or pharmacodynamic in approach. Thus, for examples, methods such as malaria-attributable fall in hematocrit (MAFH) [1,2], time to recovery from the associated anemia [14,16] and fall in hematocrit per 1000 parasites cleared from peripheral blood [17] have been applied to increases or decreases in hematocrit following treatment of acute infections with antimalarial drugs.

In recent studies, pharmacokinetic approaches such as estimation of area under curve of hematocrit deficit from 30% *versus* time (AUC) and half times of the deficit in hematocrit from 30%, the lower threshold of normal, have been used to evaluate recovery from malaria-associated anemia after antimalarial drug treatments [18,19]. There is currently no pharmacokinetic model for the evaluation of the increases in hematocrit in both anemic and non-anemic malarious children following artemisinin-based combination treatments (ACTs).

In the present study, in a group of children, we evaluated the increases in hematocrit following treatments with artesunate-amodiaquine (AA) or artemether-lumefantrine (AL), using

***Corresponding author:** Akintunde Sowunmi, Department of Clinical Pharmacology, University College Hospital, Ibadan, Nigeria, Tel: +2348023359390; Email: akinsowunmi@hotmail.com

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pharmacokinetic principles for the one-compartment constant rate intravenous infusion of drugs (CRIVID). The main objectives were: 1. to adapt the pharmacokinetic principles for constant rate intravenous infusion of drugs for the evaluation of the increases in hematocrit following AA or AL treatments of acute falciparum malaria, 2. to compare the pharmacokinetic parameters derived from the adapted model following AA or AL treatments of acute falciparum malaria in children, and 3. evaluate and compare the pharmacokinetic parameters from the adapted model in anemic and non-anemic children following treatment of acute infections with AA or AL.

Methods

Development of constant rate intravascular infusion model (CRIVIM)

Analogy between constant rate intravenous infusion of drugs and hemopoiesis and release of red blood cells into circulation: In the development of the one-compartment CRIVIM, the following assumptions, based on the pharmacokinetic principles of constant rate intravenous infusion (CRIVID), were made (Figure 1), namely:

- The bone marrow where hemopoiesis occurs is analogous to the infusion bottle or bag, and the red blood cells produced and released into the circulation, the drug.
- The rate of release of red blood cells into the circulation and the subsequent increase in hematocrit or hemoglobin is constant in any individual following treatment of falciparum malaria but the rate varies between individuals.
- Irrespective of baseline hematocrit, hematocrit value when treatment began is zero in all patients.

Assessment of pharmacokinetic parameters: Assessment from hematocrit data: (i). Hematocrit plateau, rate of rise, and clearance. An assumption is that, hematocrit or hemoglobin concentration or value is analogous to drug concentration in plasma. Consider, for a moment, that hematocrit or hemoglobin measurements before treatment and during follow-up are available for analysis. If the general equation for the relationship between plateau or steady state concentration (C_{ss}) and infusion rate of drug in a one-compartment CRIVID is:

$$C_{ss} = \frac{R_{inf}}{CL} \quad \text{..... Equation 1 [20].}$$

Where R_{inf} is infusion rate of the drug, and CL is clearance of the drug, the equation applied to plateau or steady state hematocrit (HCT_{ss}) can be written as:

$$HCT_{ss} = \frac{\text{Rate of rise in hematocrit /day}}{\text{Clearance}} \quad \text{..... Equation 2}$$

Symbol-wise, this equation can be written as:

$$HCT_{ss} = \frac{R_{incHCT}/day}{CL} \quad \text{..... Equation 3}$$

and may be interchanged accordingly. Thus:

$$R_{incHCT} = \frac{HCT_{ss}}{CL} \quad \text{..... Equation 4}$$

If baseline hematocrit when treatment began is assumed to be zero, for example, if hemetocrit at enrolment (ie. Day 0) was 20% [HCT_0 = 20%], and on each of days 21 and 28, 27%, then HCT_{ss} is 7%.

Equation 2 can then be written as:

$$HCT_{ss} = \frac{HCT_{21} - HCT_0}{\text{Time interval to } HCT_{ss} \times CL} \quad \text{..... Equation 5}$$

If HCT_{ss} is already known, in this case 7%, then CL is 7/21 = 0.33. The unit of CL is %/day. Thus CL is 0.33%day^{-1}.

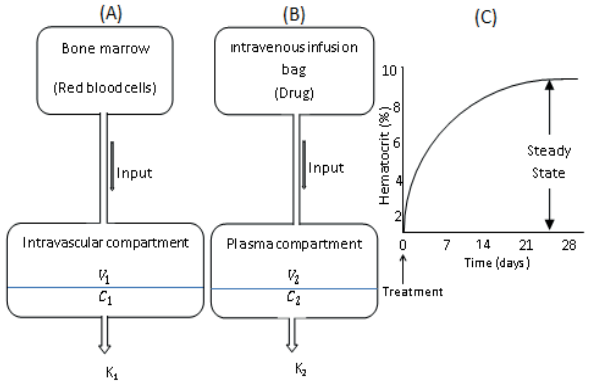


Figure 1: Hematocrit (or red blood cell) intravascular infusion analogy of a one-compartment constant rate intravenous infusion of drug. The bone marrow (A), where the red cells are made, represents the infusion bag/bottle (B). The red blood cells made (measured as hematocrit/ haemaglobin) represent the drug. Hematocrit (HCT) is assumed to be zero when antimalarial treatment begins (C). When hematocrit (or drug) input matches elimination, a steady state is reached. This level, for hematocrit, represents pre-infection hematocrit (usually hematocrit value 21-42 days after treatment begins in successfully treated patients). V_1 represents the volume into which the 'newly' released red cells are distributed and C_1 the concentration of the newly released red cells. V_2 and C_2 represent volume and concentration of drugs respectively. Plots represent increases in hematocrit from baseline versus time.

But clearance is expressed as the volume of plasma cleared of the drug per unit of time,

Therefore, it is preferable to convert hematocrit to hemoglobin which is expressed in g/dL by dividing hematocrit value by 3 [21]:

$$CL(L/day) = \frac{CL(\%/day)\times 10}{3} \quad \text{..... Equation 6}$$

- This method should be preferred for estimating clearance of red blood cells or hematocrit released into the circulation since HCT_{ss} can be determined with great precision from the temporal increases in hematocrit following treatment. Plateau hematocrit or HCT_{ss} can readily be determined by also averaging those HCT values that clearly lie at the plateau and the time interval to plateau assumed as the first or the earliest of these times.

Half-time

The half-time is easily determined, being the time taken to reach half the plateau concentration. This translates to time taken to half HCT_{ss} (in our example, half of 7%).

An accurate method of estimating the half-time uses all data obtained during follow-up, that is, from commencement of treatment to day 28 in our example. Consider, for a moment, the hematocrit concentration-time profile shown below:

Time (days) after treatment began	0	1	2	3	7	14	21	28
Hematocrit (%)	20	21	22	23	25	26	27	27
Increase in hematocrit from baseline	0	1	2	3	5	6	7	7

Figure 2 shows the plot of hematocrit *versus* time. Time to reach half HCT_{ss} must lie between 3 and 7 days, and it is approximately 5 days

since no samples were taken during this time interval. Thus one must interpolate between the observed data.

In constant rate intravenous infusion [20],

$$C_{ss} - C = C_{ss}^{e-kt} \quad \text{..... Equation 7}$$

where C_{ss} is steady state plasma concentration of the drug, and C plasma concentrations at time intervals before C_{ss} was reached.

Therefore in the constant rate intravascular infusion model,

$$HCT_{ss} - HCT_t = HCT_{ss}^{e-kt} \quad \text{..... Equation 8}$$

where HCT_{ss} is steady state hematocrit, and HCT_t hematocrit at time intervals before HCT_{ss} was reached.

The equation above on taking logarithms yields

$$\log(HCT_{ss} - HCT_t) = \log HCT_{ss} - {}^{kt}/_{2.3} \quad \text{..... Equation 9}$$

In the example in the above table, if the rate of increase in hematocrit is assumed to be constant during recovery from acute *falciparum* malaria, the decline obtained by plotting the difference between hematocrit plateau value, that is, on day 21, and that at earlier times, that is, on days 0-21, against the corresponding times on semi-logarithm paper should be a straight line (Figure 3). The intercept at time zero is the plateau concentration, that is, HCT_{ss}, and the slope is –k/2.3. The estimated half time using this method is 4.25 days (Figure 3).

Determination of volume into which the increase in hematocrit is distributed

Following constant rate intravenous infusion of a drug, the body volume into which a drug is distributed may be determined by any of the following equations [20]:

$$V_d = \frac{C_{ss}}{k} \quad \text{..... Equation 10}$$

or

$$V_d = \frac{\text{Amount of drug in body}}{k} \quad \text{..... Equation 11}$$

or

$$V_d = \frac{\text{Clearance}}{k} \quad \text{..... Equation 12}$$

Where V_d is volume of distribution, and k is the elimination rate constant of the drug.

Consider, for a moment, the theoretical volume into which the increase in hematocrit is distributed after treatment started, if it were an exogenous and not an endogenous substance. If the unit for measuring Vd is in litre (L) or L/kg, again, it is necessary to convert hematocrit which is measured in %, to hemoglobin which is measured in g/dL. Thus, the volume of distribution can be calculated in 2 simple steps viz:

(i). Convert hematocrit value (%) to hemoglobin (g/dL) by dividing hematocrit value by 3 [21].

(ii). Determine k from semi-log plot of hemoglobin concentration *versus* time (Figure 4). The value of k is the same as that for hematocrit shown in Figure 3.

Therefore the equation for determining V_d (in litre) in the model transforms simply to:

$$V_d = \frac{HCT_{ss} \times 10}{3 \times k} \quad \text{..... Equation 13}$$

Concept of hematocrit load (or burden) from start of treatment until plateau level is reached or after

In order to determine the extent of hematocrit increase from time treatment started until steady state is reached, it is essential to integrate both the plateau hematocrit and time taken to plateau, in a manner analogous to determining the area under curve (AUC) of the plot of drug concentration *versus* time. Thus, the extent of hematocrit load can be determined by numerical estimation, using trapezoidal rule, of the area under curve of the plot of the increase in hematocrit from baseline *versus* time until a plateau is reached, in a manner analogous to that of determining the area under curve after drug administration by trapezoidal rule [20,22] (Figure 5). The unit of measurement is %.day or g/dL.day if hematocrit or hemoglobin values were used. The AUC can also be determined by computer-assisted method. In our example, determination by both methods above gave similar values (107%.day).

Studies in patients

Studies on validation of the model were conducted between November 2010 and November 2013, in Ibadan, Nigeria. The studies were part of larger and longer studies of the efficacy of AA and AL in children aged 6-180 months (Pan African Clinical Trial Registry PACTR201508001188143 & PACTR201508001191898). The details of the studies have been reported elsewhere [2,14,19]. The study protocol was approved by Ministry of Health, Ibadan and the National Health Research Ethics Committee, Abuja, Nigeria.

Children aged 6-180 months presenting with acute, symptomatic, uncomplicated *falciparum* malaria, parasitemia of ≥ 2,000/μL and written informed consent given by parents or guardians were enrolled in the studies. Briefly, enrolled patients were randomized to receive AL or AA (co-formulated or co-packaged). AL (Coartem ®, Novatis, Basel, Switzerland) was given as follows: patients weighing 5-14 kg received one tablet, those weighing 15-24 kg received two tablets, those weighing 25-34 kg received three tablets, and those weighing more than 34 kg received four tablets at presentation (0 hour), 8 hours later and at

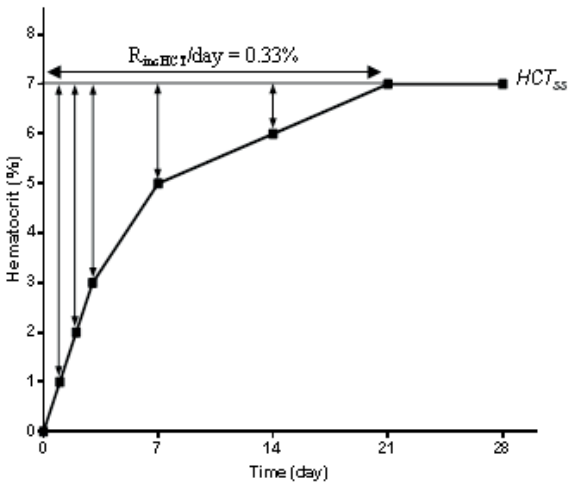


Figure 2: Estimation of pharmacokinetic parameters from hematocrit data during and after artemisinin-based combination treatments. The vertical arrows represent the difference between plateau hematocrit (HCT_{ss}) and hematocrit observed during treatment. Irrespective of baseline hematocrit, hematocrit value when treatment began was assumed to be zero.

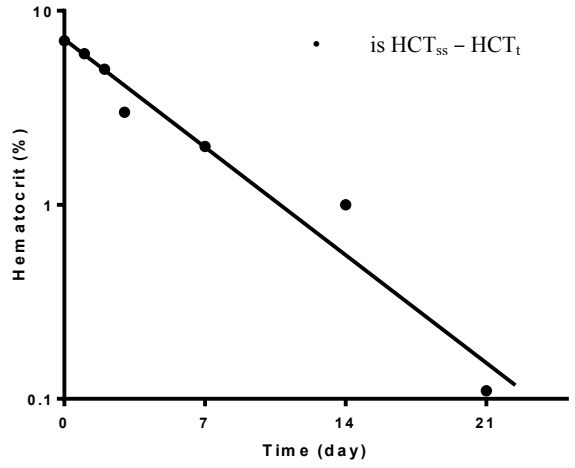


Figure 3: Semilogarithm plot of the difference between the plateau hematocrit value (HCT_{ss}) and that observed during and after treatment, against time (HCT_{ss}, hematocrit at steady state; HCT_t, hematocrit at time, t)

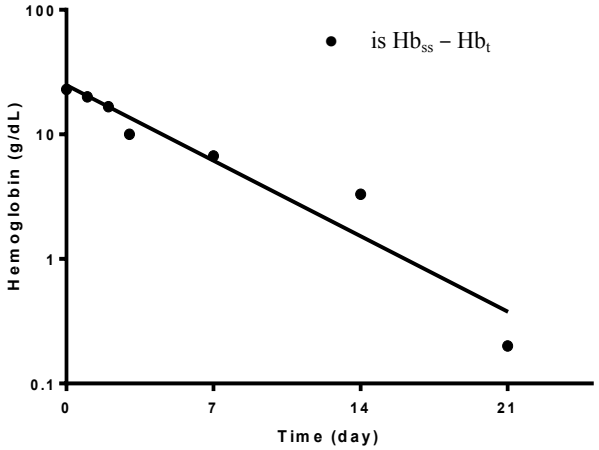


Figure 4: Semilogarithm plot of the difference between the plateau hemoglobin values (Hb_{ss}) and that observed during and after treatment against time

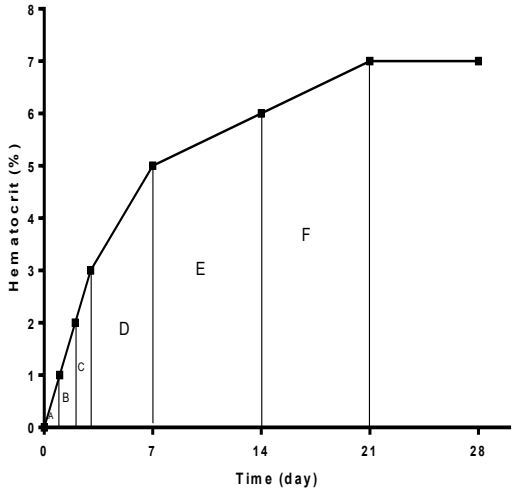


Figure 5: Plot of concentration-time data of values given in text and determination of hematocrit load by trapezoidal rule

24, 36, 48 and 60 hours after the first dose. Each tablet of AL contains 20 mg of artemether and 120 mg of lumefantrine. AA co-formulated (Coarsucam®, Sanofi Aventis, France) was given as follows: patients weighing ≥4.5 to <9 kg received one tablet, those weighing ≥9 to <18 kg received one tablet of 25 mg/67.5 mg, 50 mg/135 mg artesunate/amodiaquine respectively, those weighing ≥18 to <36 kg received one tablet and those weighing ≥36 kg received two tablets of 100 mg/270 mgin a fixed dose combination of artesunate/amodiaquine, respectively.

AA co-packaged (Dart®; Swiss Pharma, Lagos, Nigeria) was given according to body weight as follows: 5-15 kg received one tablet each of artesunate and amodiaquine, 16-24kg received two tablets each of artesunate and amodiaquine and 25-34 kg received three tablets each of artesunate and amodiaquine. Each tablet of artesunate contains 50mg and each tablet of amodiaquine contains 153 mg base in a copacked unit. All AA doses were given daily for three days. The day treatment began was taken as day 0. All drugs were given orally. Thick and thin blood films were obtained from each child as soon as they came to the clinic and the slides were carefully labeled with the patients' codes and air-dried before being stained. Follow-up with clinical and parasitological evaluation was done daily on days 1-3 and on days 7, 14, 21, 28, 35 and 42.

Asexual parasitemia in thick films were estimated by counting asexual parasites relative to 500 leukocytes, or 500 asexual forms whichever occurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6000/μL of blood. A slide was considered parasite negative if no asexual or sexual parasite was detected after examination of 200 microscope fields.

Capillary blood, collected before and during follow-up, was used to measure hematocrit using a micro-hematocrit tube and micro-centrifuge (Hawksley, Lancing, UK) on the following days 0, 1, 2, 3, 7, 14, 21, 28, 35 and 42. Anemia was defined as a hematocrit < 30% and further classified as mild, moderate or severe if hematocrit was 21-29, 15-20 or < 15%, respectively [1,2]. Anemia recovery time (in anemic patients) defined as time elapsing from commencement of drug administration to attainment of a hematocrit value ≥ 30% [14,16]. Deficit in hematocrit from 30% (in anemic patients) defined as the difference between a hematocrit value below 30% and that of 30%, considered to be the lower threshold of normal [18,19]. Patients were classified into 2 groups based on their enrolment hematocrit value: < 30% and ≥ 30%. Patients were evaluated in the model if the following criteria were met: hematocrit was measured at all visits (from enrolment till day 42), and a minimum of 2-3 increases were recorded before a steady state value was reached.

Response to drug treatment was assessed using a modified version of the World Health Organization 2003 *in vivo* clinical classification criteria [23] as previously described [24]. Cure rates were defined as the percentages of patients whose asexual parasitemia cleared from peripheral blood and who were free of patent asexual parasitemia on days 14, 21 and 28 of follow-up. The cure rates on days 21 and 28 were adjusted on the basis of the polymerase chain reaction (PCR) genotyping results of paired samples of patients with recurrent parasitemia after day 7 of starting treatment.

Data analysis

Data were analyzed using version 6 of Epi-Info software [25] and the statistical program SPSS for Windows version 20.0. [26].

Variables considered in the analysis were related to the densities of *P. falciparum* asexual (or the presence of sexual forms). Proportions were compared by calculating χ^2 using Yates' correction, Fisher exact or Mantel Haenszel tests. Normally distributed, continuous data were compared by Student's t test and analysis of variance (ANOVA). Data not conforming to a normal distribution were compared by the Mann-Whitney U tests and the Kruskal Wallis tests (or by Wilcoxon ranked sum test). The association between two continuous variables was assessed by Pearson's or Spearman's rank correlation coefficient. Agreement between anaemia recovery time and haematocrit half-time was assessed by Bland-Altman analysis [27]. Values of P <0.05 were taken to indicate significant differences. Data were double entered serially using patients' codes and were only analyzed at the end of the study.

Results

Patients' characteristics

Of 248 children who had hematocrit values taken before and during the entire 6 weeks of follow-up, 112 children had increases in hematocrit on at least 2 occasions before a plateau or the beginning of a plateau was reached. The temporal changes in hematocrit following treatment in the 248 children have been reported elsewhere [19]. Eighty one of these children were treated with AA and 31 with AL (an approximate ratio of 3:1 of the children enrolled during the trial period). The characteristics of children and treatment outcomes are summarized in Table 1. Fifty seven children had a hematocrit < 30% at presentation. The characteristics and treatment outcomes were similar in both AA and AL treatment groups. The proportions of children with increases in hematocrit following treatments were significantly higher in those with hematocrit <30% compared to ≥30% at presentation (57 of 63 [90%] *versus* 55 of 185 [30%], P < 0.0001).

Recovery from anemia

Anemia was mild or moderate in 53 or 4 children, respectively. All children (57) recovered from their anemia by day 21; 13, 23 and 15 children recovered from their anemia by days 7, 14 and 21, respectively. Mean anemia recovery time was 13.8 days (Range, 1 – 21; 95% CI 11.9 – 15.7).

Kinetics of the increases in hematocrit and of declines in hematocrit after treatment

Parameter	AA (*n* = 81)	AL(*n* = 31)	ALL(*n* = 112)	*P* value
Gender (M/F) **Age** (years)	47/34	16/15	63/49	0.54
Mean (sd)	6.5(3.1)	5.2(2.6)	6.1(3.0)	0.05
Range	0.7-14	0.8-13	0.7-14	
No. <5yrs	29	18	47	0.03
Weight (kg)				
Mean (sd)	18.3(6.0)	15.3(4.5)	17.5(5.8)	0.01
Range	7-36	7-25	7-36	
Duration of illness (day)				
Mean (sd)	2.8(1.3)	2.8(0.7)	2.8(1.2)	0.92
Range	1-7	1-4	1-7	
Temperature (°C)				
Mean (sd)	38.3(1.1)	38(1.2)	38.1(1.1)	0.39
Range	35.9-40.5	35-40.5	35-40.5	
No. with >37.4°C	63	24	87	0.97
No. with >40°C	4	2	6	0.75
Hematocrit (%)				
Mean (sd)	30.1(4.6)	29.2(4.1)	30(4.5)	0.34
Range	20-38	18-37	18-38	
No. with <30%	39	18	57	0.47
Parasitemia (μL^{-1})				
Geometric mean	59,727	62,433	60,460	0.85
Range	2,094-346,153	7,588-288,462	2,094-346,153	
No. with >100,000μL^{-1}	21	6	27	0.47
No. with >250,000μL^{-1}	4	2	6	0.75
Parasite clearance time (day)				
Mean (sd)	1.1(0.3)	1.1(0.3)	1.1(0.3)	0.38
Fever clearance time (day)				
Mean (sd)	1(0.1)	1.2(0.4)	1.1(0.2)	0.01
Cure rate on day 42 (%)	98	97	97	0.83*

Table 1: Demographic characteristics at enrolment, and treatment outcomes of children treated with AA or AL.AA: artesunate-amodiaquine; AL: artemether-lumefantrine; ALL: all children; *: Mantel Haenszel test.

Overall, there was a progressive increase in hematocrit above baseline following treatment such that a steady state hematocrit of 6.7% (95%CI 5.9-7.5) was reached at a median time of 28 days (95%CI 23.2-26.8) (Figure 6). Plateau hematocrits were similar in children treated with AA and AL (Table 2). Overall, declines in hematocrit were mono-exponential (Figure 7) with estimated mean half-time ($t_{½}$) of 2.5 days (95%CI 2.2-2.8). Half-times and volumes of distribution were similar in children treated with AA or AL (Table 2).

Comparison of kinetics of increases in hematocrit and of the declines in hematocrit following ACTs in anemic and non-anemic children

Overall, following successful treatment of the infections, there were increases in hematocrit from baseline in both anemic and non-anemic children (Figure 8). Plateau hematocrits were significantly lower in non-anemic compared to anemic children [4.9% (95%CI 4.2-5.6) *versus* 8.2% (95%CI 7.1-9.4), P < 0.0001; Table 3]. Median time to reach plateau hematocrit was also significantly shorter in non-anemic compared with anemic children [21 days (95%CI 19.4-24.8) *versus* 28 days (95%CI 25.2-29.8), P = 0.001; Table 3]. Plateau hematocrit values and median times to reach the plateau values were similar in anemic children treated with AA or AL [8.7% (95%CI 7.2-10.2), n = 39 *versus* 7.3% (95%CI 5.3-9.2) n = 18; P = 0.23 and 28 days (95%CI 25.7-31.1) *versus* 28 days (95%CI 21.3-30.1), P = 0.18, respectively]. Also plateau hematocrits and median times to reach plateau were similar in non-anemic children treated with AA or AL [5.1% (95%CI 4.3-5.9) n = 42 *versus* 4.8% (95%CI 2.8-6.8) n = 13, P = 0.7 and median 21 days (95%CI 19.4-25.4) *versus* median 21 days (95%CI 14.1-28.2), P = 0.76, respectively]. In anemic children on days 2, 7, 14, 21, 28 and 35, 39%, 55%, 76%, 86%, 97% and 100% of plateau heamatocrit, respectively, was reached following treatment. In non-anemic children on days 2, 7, 14, 21, 28 and 35, 41%, 61%, 74%, 86%, 96% and 100% of plateau heamatocrit, respectively, was reached. These parameters were similar in anemic and non-anemic children.

Overall, declines in hematocrit were mono-exponential in children presenting with and without anemia (Figure 9). Half-times were significantly shorter in anemic compared to non-anemic children (Table 3). Estimated mean half-times were similar in anemic children

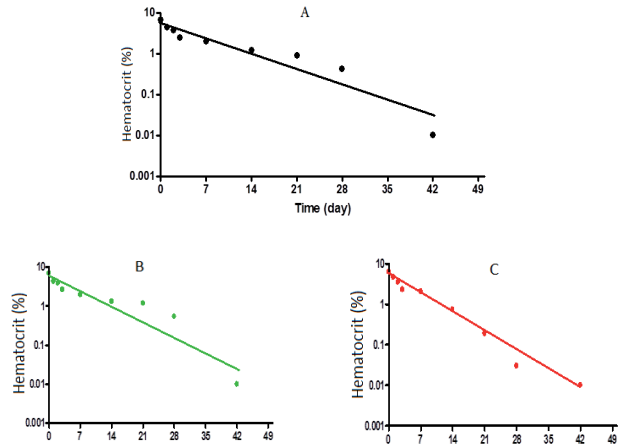
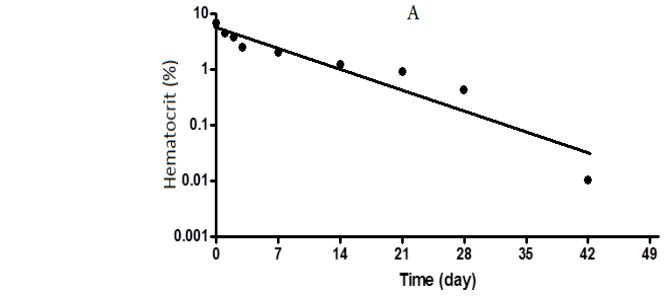


Figure 6: Approach to plateau hematocrit after treatment of acute falciparum infections with artesunate-amodiaquine or artemether-lumefantrine. (green line, artesunate-amodiaquine; red line, artemether-lumefantrine; black line, All children)

Parameter	Artesunate-amodiaquine (*n* = 81)	Artemether-lumefantrine (*n* = 31)	ALL (*n* = 112)	*P* value
HCTss (or Css) [%]				
Mean	6.8	6.3	6.7	0.56
95%CI	6-7.7	4.9-7.7	5.9-7.5	
THCTss (or Tss) [day]				
Median	28	28	28	0.49
95%CI	23.3-27.5	20.1-27.6	23.9-27.5	
Rate of increase in HCT per day(%/day)				
Median	0.3	0.2	0.3	0.62
95%CI	0.3-0.4	0.1-1	0.2-0.6	
Clearance (/day)				
Median	0.04	0.04	0.04	0.49
Range	0.03-1	0.03-1	0.03-1	
$T_{½}$ [day]				
Mean	2.4	2.7	2.5	0.46
95%CI	2.1-2.8	2-3.3	2.2-2.8	
$AUC_{0\text{-}HCTss}$ [%.d]				
Mean	86.6	58.1	78.2	0.12
95%CI	65.5-107.6	34-82.3	61.7-94.6	
$AUC_{0\text{-}42d}$ [%.d]				
Mean	89.9	65.9	82.8	0.17
95%CI	69.5-110	42.5-89.3	66.9-98.7	
$AUC_{0\text{-}HCTss}$:$AUC_{0\text{-}42d}$				
Mean	1.4	3	1.9	0.10
95%CI	1.2-1.6	0.02-6	1-2.8	
Rate of increases in $AUC_{0\text{-}HCTss}$ [%]				
Mean	2.8	2.3	2.7	0.28
95%CI	2.3-3.5	1.5-3.1	2.2-3.2	
Vd (L/kg)				
Mean	4.6	5.1	4.8	0.57
95%CI	3.7-5.6	3.7-6.5	4-5.5	

Table 2: Measures of steady state parameters in children treated with artesunate-amodiaquine or artemether-lumefantrine.HCT: hematocrit; HCTss: hematocrit at steady state; THCTss: time to steady state; $T_{½}$: half-time of increases to or elimination from steady state; Vd, volume of distribution of the increases; $AUC_{0\text{-}HCTss}$: area under curve of increases from time zero (commencement of treatment) to time of steady state; $AUC_{0\text{-}42d}$: area under curve of increases from time zero (commencement of treatment) to 42 days; ALL: all children.

treated with AA or AL [2.1 days (95%CI 1.7-2.5) *versus* 2.1 days (95%CI 1.5-2.7), *P* = 0.83]. Estimated half-times were also similar in non-anemic children treated with AA or AL [2.4 days (95%CI 2.1-2.8) *versus* 2.7 days (95%CI 2-3.3), *P* = 0.46]. The AUCs, the rates of increases in AUCs were significantly higher in anemic than in non-anemic children (Table 3). However, the ratio of $AUC_{0\text{-}HCTss}$: $AUC_{0\text{-}42d}$ and the volume of distribution were similar in anemic and non-anemic children (Table 3). The proportions of children with rates of increases in areas under



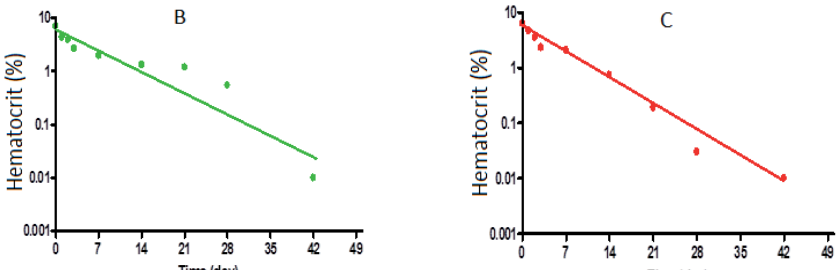


Figure 7a: Semilogarithm plots of the difference between the plateau hematocrit and that observed during and after treatment in all children. **Figure 7b and c:** Semilogarithm plots of the difference between the plateau hematocrits and that observed during and after treatment with artesunate-amodiaquine (B) and artemether-lumefantrine (C)

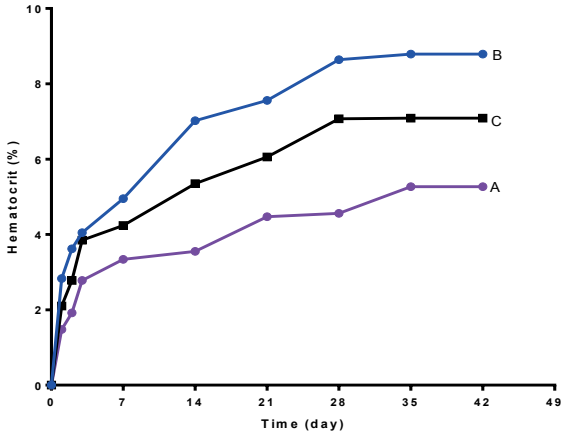


Figure 8: Approach to plateau in children with ≥ 30% (A) or <30% (B) hematocrit following artemisinin-based treatments and in all children (C)

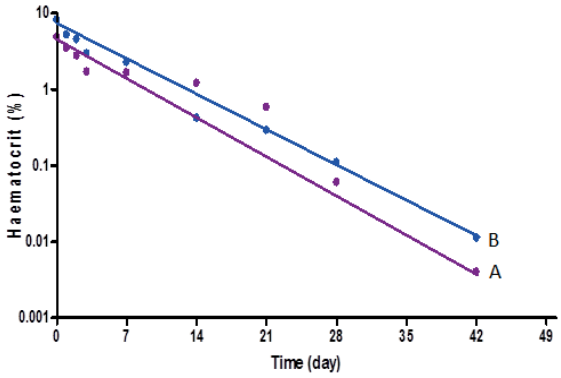


Figure 9: Semilogarithm plots of the difference between the plateau hematocrit and that observed following treatment in children with hematocrit ≥30% (A) and <30% (B).

curves greater than 3% per day was significantly higher in anemic compared to non-anemic children (30 of 57 [53%] *versus* 2 of 55 [4%], respectively; $\chi^2 = 32.6$; $P < 0.0001$).

Relationship between deficit in hematocrit and areas under curves of increases in hematocrit *versus* time in children with anemia

Data for deficit in hematocrit from 30% and areas under curves were available in 57 children who were anemic at presentation. Overall, there was a significantly positive correlation between deficit in hematocrit from 30% and area under curve of increase in hematocrit to steady state *versus* time ($AUC_{0\text{-}HCTss}$) [$r = 0.497$, $P < 0.0001$]. Similarly, there was a significantly positive correlation between deficit in hematocrit from 30% and area under curve of increase in hematocrit at 42days *versus* time ($AUC_{0\text{-}42d}$) [$r = 0.514$, $P < 0.0001$]. However, there was no correlation between excess in heamatocrit above 30% at enrolment and areas under curves of increase in hematocrit *versus* time in non-anemic children ($r = 0.071$, $P = 0.79$).

Relationship between anemia recovery time and half-time of hematocrit (anemia) in the same patients

The relationship between the half-time of decline in hematocrit deficit from plateau and anemia recovery time in the same patients with anemia at presentation was evaluated in 57 children. The mean half-time of decline in hematocrit deficit from plateau hematocrit was 2.5 days (95% CI 2.2 – 2.8). The mean anemia recovery time was 13.8 days (95% CI 11.9 – 15.7). Bland-Altman plots of the anemia recovery times

Parameter	Hematocrit ≥30% followed by increase [Pattern 1] (*n*=55)	Hematocrit <30% followed by increase [Pattern 4] (*n*=57)	ALL	*P* value
Body weight (kg)				
Mean	19.3	15.8	17.5	0.001
Range	7-36	8-26	16.4-18.6	
tAnemia recovery time [day]				
Mean	-	13.8	13.8	-
95%CI		11.9-15.7	11.9-15.7	
HCTss (or Css) [%]				
Mean	4.9	8.2	6.7	<0.0001
95%CI	4.2-5.6	7.1-9.4	5.9-7.5	
THCTss (or Tss) [day]				
Median	21	28	28	0.001
Range	19.4-24.8	25.2-29.8	23.9-27.5	
Rate of increase of HCT per day(%/day)				
Median	0.2	0.3	0.3	0.23
Range	0.06-2	0.06-11.5	0.1-11.5	
Clearance (/day)				
Mean	0.05	0.04	0.04	0.001
Range	0.03-1	0.03-1	0.03-1	
$T_{½}$ (day)				
Mean	2.9	2.1	2.5	0.01
95%CI	2.4-3.5	1.8-2.4	2.2-2.8	
$AUC_{0\text{-}HCTss}$ [%.d]				
Mean	24.5*	110.9*	78.2	<0.0001
95%CI	14-35	89-132.9	61.7-94.6	
$AUC_{0\text{-}42d}$ [%.d]				
Mean	30.1	117.7	82.8	<0.0001
95%CI	20.4-39.7	96.2-139.2	66.9-98.7	
$AUC_{0\text{-}HCTss}$:$AUC_{0\text{-}42d}$				
Mean	1.8	1.9	1.9	0.91
95%CI	1.4-2.2	0.5-3.3	1-2.8	
Rate of increases in $AUC_{0\text{-}HCTss}$ [%]				
Mean	1.0	3.7	2.7	<0.0001
95%CI	0.7-1.3	3.1-4.3	2.2-3.2	
Vd (L/kg)				
Mean	4.1	5.4	4.8	0.10
95%CI	2.9-5.2	4.3-6.5	4-5.5	

Table 3: Measures of steady state parameters in children with hematocrit ≥30% or <30% followed by increases.HCT: hematocrit; HCTss, hematocrit at steady state; THCTss: time to steady state; $T_{½}$: half-time of increases to or elimination from steady state; Vd: volume of distribution of the increases; $AUC_{0\text{-}HCTss}$: area under curve of increases from time zero (commencement of treatment) to time of steady state; $AUC_{0\text{-}42d}$: area under curve of increases from time zero (commencement of treatment) to 42 days; ALL, all children*: Ratio of $AUC_{0\text{-}HCTss}$ in anemic children to $AUC_{0\text{-}HCTss}$ in non-anemic child was approximately 4.5:1**: Ratio of $AUC_{0\text{-}42d}$ in anemic children to $AUC_{0\text{-}42d}$ in non-anemic child was approximately 4:1.

and multiples of hematocrit half-times are shown in Figure 10. The limits of agreement between anemia recovery times and 7 or 8 multiples of hematocrit half-times were narrow and insignificant (-13.8 – 12.3 days and -15.9 – 11.1 days, P = 0.17 and 0.68, respectively). The limits of agreement were not narrow and were significant at 9 or 10 multiples of hematocrit half-times (Figure 10).

Relationship between parasitemia and measures of steady state parameters

Overall, steady state kinetic parameters were independent of pre-treatment parasitemia (Table 4). However, the areas under curves were significantly higher at higher parasitemias in excess of 100,000μL^{-1} compared with lower parasitemias.

Discussion

In this study, the principles and applications of the variations in drug concentrations with time following one-compartment CRIVIDs have been adapted to describe and analyse the variations in increases in capillary hematocrit from baseline with time, following successful treatment of acute falciparum infections with AA or AL. The assumptions that hematocrit value at the time treatment started was zero and the rate of increase in hematocrit was constant in any individual permitted close analogy to the basic concepts of one compartment CRIVID. In this regard, the criterion that an increase in hematocrit for at least 2-3 times before plateau heamatocrit was reached, before inclusion into the analyses led to the exclusion of a third of all patients enrolled. Thus without increases with time, it was difficult to employ the one-compartment CRIVIM.

Increases in hematocrit may occur in non-anemic and anemic children following ACTs of acute uncomplicated falciparum infections. Twenty nine percent and 94%, respectively of children in these two categories were eligible for evaluation using the CRIVIM.

These proportions were expected since a large proportion of mild to moderately anemic children are expected to recover from their acute non-severe malaria-associated anemia. In this regard, anemia recovery time of approximately 14 days was similar to that recently reported in a large study of under 5 year old malarious Nigerian children following ACTs [16].

Recovery from uncomplicated falciparum malaria-associated declines in hematocrit is complete when, after complete elimination of the parasites, and in the absence of other complications, hematocrit or hemoglobin levels return to pre-infection levels. This process takes approximately 4-6 weeks after the start of successful treatment of the infections [1,2]. In CRIVIM, the pharmacokinetic equivalent of this dynamic process is the approach to plateau hematocrit following successful treatment of the infections. Thus in CRIVIM, plateau hematocrit was reached when red blood cell production matched red blood cell loss. The equivalent of the process in a one-compartment CRIVID is when the rate of infusion of a drug matches the rate of elimination of the drug [20].

Parameter	<50,000 μL^{-1} (*n*=40)	50,000 –100,000μL^{-1} (*n*=44)	>100,000μL^{-1} (*n*=28)	ALL	*P* value
Hematocrit (%)					
Mean	30.4	29.9	28.9	29.8	0.41
95%CI	28.9-31.8	28.5-31.4	27.1-30.6	29-30.7	
HCTss (or Css) [%]					
Mean	6.2	6.3	7.8	6.7	0.26
95%CI	5.2-7.2	5.1-7.75	5.9-9.8	5.9-7.5	
THCTss (or Tss) [day]					
Median	28	28	28	28	0.33
95%CI	21.7-27.7	23.6-29.1	18.3-26.9	23.9-27.5	
$T_{½}$ (day)					
Mean 95%CI	2.7 2.2-3.3	2.7 2.13-3.2	1.9 1.5-2.4	2.5 2.2-2.8	0.11
$AUC_{0\text{-}HCTss}$ [%.d]					
Mean	48.6	88.2	107.9 65.1-150.6	78.2 61.7-94.6	0.017
95%CI	28.5-64.6	34-82.3			
$AUC_{0\text{-}42d}$ [%.d]					
Mean	56.3	88.4	113	82.8	0.024
95%CI	37-75.6	61.5-89.3	72-154	66.9-98.7	
$AUC_{0\text{-}HCTss}$:$AUC_{0\text{-}42d}$					
Mean	1.3	1.3	1.6	1.9	0.36
95%CI	0.3-5.2	1-1.6	1.1-2.1	1-2.8	
Vd (L/Kg)					
Mean	5.1	5.0	4.0	4.8	0.56
95%CI	3.8-6.3	3.42-6.66	3.1-4.9	4-5.5	
Haemotocrit deficit from 30%					
Mean	3.6	4	3.6	3.7	0.88
95%CI	2.2-4.9	2.6-5.4	2.3-4.9	3-4.5	
No.	19	20	18	57	0.18

Table 4: Measures of steady state parameters in children with enrolment parasitemia < 50,000, 50,000-100,000 and >100,000μL^{-1}.
HCT: hematocrit; HCTss: hematocrit at steady state; THCTss: time to steady state; $T_{½}$: half-time of increases to or elimination from steady state; Vd: volume of distribution of the increases; $AUC_{0\text{-}HCTss}$: area under curve of increases from time zero (commencement of treatment) to time of steady state; $AUC_{0\text{-}42d}$: area under curve of increases from time zero (commencement of treatment) to 42 days; ALL: all children.

The similar approach to plateau, time to plateau and the plateau hematocrit following treatment with AA or AL suggest both drugs are equivalent in their effects on uncomplicated malaria-associated increases in hematocrit in children from this endemic area. The median time to plateau of 28 days after treatment started suggests the pharmacokinetics equivalent of the pharmacodynamic process is reached in approximately the same time of four weeks after start of treatment. This is approximately nine half-times and > 99% of plateau was reached at this time (see below).

Approach to plateau and the plateau hematocrit values differ considerably in anemic and non-anemic children in the following ways: plateau hematocrit was significantly higher and time to reach plateau hematocrit was significantly shorter in anemic children compared with non-anemic children. These differences would suggest that the rate of rise in hematocrit following treatment was accelerated in anemic children compared with non-anemic children thus, compensating for the greater loss of hematocrit (or red blood cells) in the anemic children. Alternatively, it may mean that the relatively rapid clearance of parasitemia following artemisinin-based combination treatments may contribute significantly to the factors responsible for the rapid recovery from malaria-associated decline in hematocrit.

It would appear increases in hematocrit are a first order process and its half-time was independent of enrolment parasitemia, the hallmark of first order process. The hematocrit half-time of 2.5 days provides a baseline for which future changes in the kinetics of increases in hematocrit following ACTs in children from this endemic area may be measured or compared.

An important concept in the development of the model is the volumes into which the increases in hematocrit were distributed. It is unusual to determine the volume of distribution of an endogenous product that is increased after treatment of an infection. However, the ability to quantify the increases and calculate half-times (or elimination rate constants) permits evaluation of the volumes into which the 'newly made red blood cells', measured as rises in hematocrit, are distributed since at steady state when red blood cell production matches elimination, volume of distribution can be determined by simple equations. The significantly larger volume of distribution of hematocrit in anemic compared to non-anemic children was not unexpected for a number of reasons: the higher steady state hematocrit in anemic children; the lower body weight in anemic children; the similar elimination rate constant, *k*, in anemic and non-anemic children. Thus it is likely that the depleted hematocrit in anemia required a large volume of distribution of the newly increased hematocrit compared to non-anemic children with relatively lesser depletion of hematocrit.

Hematocrit burden following treatment was quantified using AUC of the plot of increase in hematocrit from baseline *versus* time as an approach to combine their duration and magnitude. Hematocrit burden was significantly higher in anemic compared to non-anemic children. Indeed, hematocrit burden was approximately 5 folds higher in anemic compared to non-anemic children. The significantly higher rate of increases in AUC in anemic children would suggest an acceleration of the process of recovery from malaria-associated decrease in hematocrit in anemic children. This finding was expected. In addition, the significant correlation between deficit in hematocrit from 30% and areas under curve suggests that areas under curves may be used as a measure of the burden of malaria-associated decreases in hematocrit in anemic children.

It would seem likely that, in the absence of significant pre-infection bone marrow dysfunction or disease, the increases in hematocrit following treatment of acute infections with AA or AL may be directly attributable to bone marrow recovery from, or the attenuation of the adverse effects of malaria infections on bone marrow functions. In this context, the rapid clearance of the parasitemia by both ACTs resulted in similar effects on the kinetics of the increase in hematocrit after treatment. This outcome was expected.

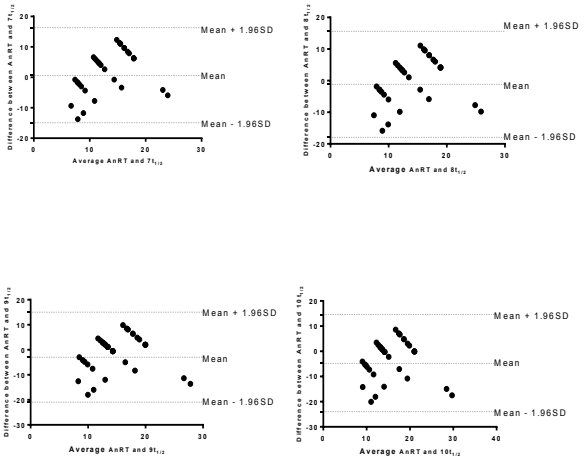


Figure 10: Bland-Altman plots of anemia recovery times and multiples [7 (A), 8 (B), 9 (C) and 10 (D)] of anemia half-times. Biases were 1.3, -0.39, -2.09 and -3.79 for plots A, B, C and D; P = 0.17, 0.68, 0.043 and 0.007, respectively. The mean values ± 1.96standard deviation (SD) of the differences are shown

Despite analogy to one-compartment CRIVID, there are obvious differences between the CRIVID and CRIVIM. An important difference is post infusion data amenable to pharmacokinetic analyses in CRIVID are not possible with CRIVIM for hematocrit increases. In the absence of immediate or post plateau infections in the individual, post plateau decline in hematocrit or hemoglobin is not possible in CRIVIM. Thus, the estimation half-time from post plateau declining curve is not feasible. However, it is plausible to expect a decline in hematocrit or hemoglobin post-infection would follow a first order process.

In conclusion, the one compartment CRIVIM permits a novel pharmacokinetic analyses of the increases in hematocrit or hemoglobin after artemisinin-based combination treatments of acute malaria infections, and can be used in observational and clinical studies involving antimalarial drugs.

Conflicts of Interests

The authors declare they have no competing interests.

Authors' Contributions

AS developed the model, led the design, conduct, data analysis and manuscript preparation. KA, AIA, GN, BF, TA and EOA participated in data collections and analysis. All authors read and approved the final draft of the manuscript.

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Nomenclature

AA: Artesunate- Amodiaquine

ACTs: Artemisinin-Based Combination Treatments

AL: Artemether- Lumefantrine

AnRT: Anemia Recovery Time

AUC: Area Under Curve

C: Plasma Concentration

CL: Clearance

CRIVID: Constant Rate Intravenous Infusion of Drug

CRIVIM: Constanat Rate Intravascular Infission Model

Css: Steady State Concentration

HCT: Hematocrit

HCT_0: Heamatocrit at presentation

HCTss: Steady State Heamatocrit

k: Rate Constant

$t_{½}$: Half-time

Vd: Volume of Distribution

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