

Reticulocyte count changes in paediatric patients with uncomplicated malaria treated with artemisinin combination therapy

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Abstract

Background: Acute malaria is associated with reticulocyte count depression, and artemisinin derivatives have also been shown to cause reversible count changes in patients with severe malaria. However, there has been no report on the effect of artemisinin on reticulocyte count when used in combination with other antimalarials, or the effect of artemisinin-based treatment on reticulocyte count changes in patients with uncomplicated malaria.

Objective: This study reports the effects of artemisinin-based antimalarial treatments on reticulocytes among paediatric patients with uncomplicated malaria.

Methods: The reticulocyte counts, haemoglobin, and platelet counts of children with uncomplicated malaria treated with artesunate-amodiaquine (116), artemether-lumefantrine (90), or amodiaquine (17) were measured before treatment (day 0) and then on days 3, 7, 14, and 28.

Results: The fractional changes in reticulocyte counts were higher in the artesunate-amodiaquine and artemether-lumefantrine groups during the initial stages of treatment. However, the overall fractional reticulocyte change between acute illness and pre-illness levels was higher in the amodiaquine treated group. There was a negative correlation between haemoglobin and reticulocyte counts before treatment (day 0) and on all the follow up days.

Conclusion: Treatment of uncomplicated malaria in children with artesunate-amodiaquine or artemether-lumefantrine was associated with less profound reticulocyte count changes compared with children treated with amodiaquine alone. These changes were most likely due to the rapid parasite clearance by the two artemisinin-based combination treatment regimens.

Keywords: Artemisinin combination therapy, reticulocytes, uncomplicated malaria

INTRODUCTION

In both animal [1] and human [2] malaria, the reticulocyte count is depressed during acute disease. Few studies have evaluated the effect of specific antimalarial treatment on reticulocyte count dynamics during acute malaria. The artemisinin derivatives which form the backbone of current malaria treatment, exhibit an excellent safety profile. However, in animals, these highly efficacious antimalarials have demonstrated both embryotoxic and reticulocytopenic effects [3]. Reticulocytes are immature red blood cells that originate from orthochromatic normoblast through nuclear exclusion in

the bone marrow, are released into the peripheral blood, and undergo further differentiation into mature red blood cells. Furthermore, in humans, the artemisinin derivatives show variable effects on a series of blood cell indices including, inhibition of lymphocyte proliferation [4] and depression of neutrophil phagocytic ability [5]. Acute malaria is associated with red blood cell destruction [6], resulting in anaemia. Also, a temporary decrease in reticulocyte counts has been demonstrated in paediatric severe malaria patients treated with artemisinin derivatives [7]. In order to determine if a similar effect occurs in uncomplicated malaria, reticulocyte counts reflecting the erythropoietic activity of the bone marrow, rate of erythrocyte delivery from the bone marrow into the peripheral blood, and the rate of reticulocyte maturation indices were measured in Ghanaian children with acute uncomplicated malaria treated with

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either artesunate-amodiaquine or artemether-lumefantrine, the two most widely used artemisinin combination therapies in sub-Saharan Africa. The indices were compared with that of a limited number of children with uncomplicated malaria treated with amodiaquine alone.

MATERIALS AND METHODS

The data reported in this paper is from a sub-study of a clinical trial that was conducted to evaluate the efficacy and safety of artemisinin-based combination therapies for uncomplicated malaria in children in Accra, Ghana [Registration number NCT 00406146, (<http://www.clinicaltrials.gov>)]. The full details of the trial have been reported elsewhere [8]. Briefly, children with uncomplicated malaria fulfilling pre-defined inclusion criteria were recruited after obtaining written informed consent from the accompanying parent or guardian. Children were randomized to receive standard therapeutic doses of (i) amodiaquine (AQ) (Camoquine®, Pfizer; Dakar, Senegal) alone; or (ii) amodiaquine in combination with artesunate (AS+AQ) (Plasmodium®, Mepha; Switzerland); or (iii) artemether-lumefantrine (AR-L) (Coartem®, Novartis Pharma AG, Basel, Switzerland). After completion of recruiting procedures, venous blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes before treatment (day 0), and on days 3, 7, 14, 28, for routine haematological investigations. Parasite counts were determined in Giemsa-stained blood films relative to 200 white blood cells (WBC) and the measured WBC count.

Reticulocyte count by microscopy

The supravital staining technique was used for reticulocyte enumeration. The EDTA sample (final concentration, 1.5 mg/mL) was stained within 2 h, with a citrate-saline/methylene blue method. The red cells were gently resuspended and conventional wedge smears were made to produce an evenly spread thin film of supravital stained sample on a microscope slide. The films were dried and viewed under a microscope for enumeration of reticulocytes.

Statistical analysis

The reticulocyte count was expressed as a percentage of red blood cells and multiplied with the measured erythrocyte number to obtain absolute reticulocyte counts. Statistical analysis was done using two-way ANOVA for repeated measurements and by multiple linear regression analyses. All $p < 0.05$ were considered statistically significant.

RESULTS

The reticulocyte counts of a total of 223 children were available for analysis (Amodiaquine, 17; Artesunate-amodiaquine, 116; Artemether-lumefantrine, 90). The reason for the low number of patients in the AQ group is the change in national policy for treatment of uncomplicated malaria from chloroquine to artesunate-amodiaquine in 2005, which made it unethical to continue treatment with monotherapy; therefore, the amodiaquine-alone arm was discontinued. The baseline demographic and selected clinical characteristics were similar between the treatment groups (Table 1). The mean baseline percentage reticulocyte count was slightly lower (though non-significantly) in the two artemisinin-based combination therapy

(ACT) groups (Table 1), however, the absolute reticulocyte counts showed an increasing trend from day 0 and peaked at day 7 for all three treatment groups (Figure 1). The fractional change in reticulocyte count in the initial days of treatment (from baseline to day 3), was higher in the two ACT groups, compared with the fractional change in amodiaquine monotherapy group (Table 2). However, the change in reticulocyte counts between baseline and day 28 (which may represent a difference between acute illness and pre-treatment or steady state levels) was higher in the AQ group, and increased in the order, artemether-lumefantrine < artesunate-amodiaquine < amodiaquine monotherapy (Table 3). There were no significant differences however between the groups pre- and post-treatment (2-way ANOVA, $p = 0.69$). The haemoglobin levels, as expected, exhibited an initial decreasing trend between day 0 and 3 in all three groups, followed by a steady rise till day 28 (Figure 2).

Table 1: Baseline characteristics of subjects

Characteristics	AQ (n=17)	AS+AQ (n=116)	AL (n=90)	<i>p</i> value
Age in yr.	7.0 (3.4)	7.0 (3.6)	7.0 (3.6)	0.54
Sex	M=7, F=10	M=63, F=53	M=48, F=42	
Haemoglobin count	11.7 (2.2)	11.2 (2.1)	11.4 (2.3)	0.67
Parasite count	48,984	62,992	56,768	0.74
Reticulocyte %	1.32	0.66	0.74	-
Platelet count	171.6 (140.2)	163.3 (125.0)	157.2 (104.6)	0.89

*AQ, Amodiaquine; AQ+AS, Artesunate-amodiaquine; AL, Artemether-lumefantrine; Data are means (standard deviation); M, males; F, females.

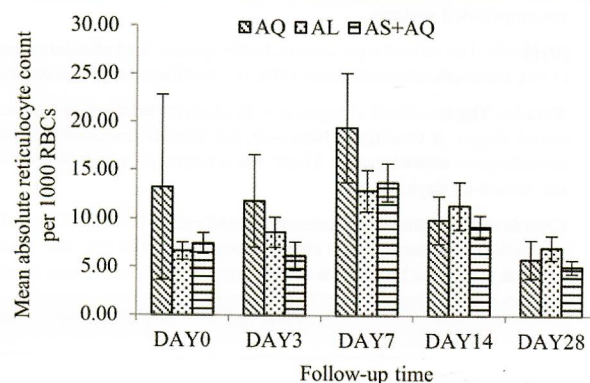


Figure 1: Mean reticulocyte counts per 1000 red blood cells

Table 2: Fractional changes in reticulocytes between baseline and day 3 of treatment in the children treated with AQ, AL or AS+AQ

Group	Mean difference (95% CI) [day 0 - day 3]	Proportional difference (per endpoint)	<i>p</i> value
AQ	1.42 (-18.97 - 21.81)	11.97%	0.89
AL	-1.96 (-5.48 - 1.56)	22.77%	0.27
AS+AQ	1.28 (-2.25 - 4.82)	20.85%	0.47

*AQ, Amodiaquine; AL, Artemether-lumefantrine; AS+AQ, Artesunate-amodiaquine; CI, confidence interval

The platelet counts were initially low, as expected, and showed a steady rise till day 14 and then a slow decrease in all 3 groups (Figure 3). There was, as expected, a negative, non-significant, correlation between haemoglobin level and reticulocyte count on all the days of measurement after baseline (day 0, $r = 0.03$, $p = 0.68$; day 3, $r = -0.08$, $p = 0.35$; day 7, $r = -0.03$, $p = 0.64$; day 14, $r = -0.08$, $p = 0.33$; and day 28, $r = -0.02$, $p = 0.79$). The correlation between admission parasite density and reticulocyte count on day 0 was not statistically significant ($r = 0.02$, $p = 0.78$). A regression analysis (with age as an independent variable and platelet or reticulocyte count as dependent variable) did not show a significant association between age and either platelet or reticulocyte counts on any of

the days of assessment (data not shown). Furthermore, the reticulocyte production index or corrected reticulocyte count (an indication of the appropriateness of the bone marrow response to the malaria-induced anaemia), was comparable between the three groups respectively [(mean $\pm$ standard deviation): AQ, 0.3 ± 0.9 vs AL, 0.3 ± 0.6 vs AS+AQ, 0.3 ± 0.6] after treatment.

DISCUSSION

The reticulocyte count is depressed in acute malaria and although artemisinin derivatives have been shown to exert a series of effects on cellular blood components, there has to date, been a single report, by Cao et al. [7], that has reported an effect of treatment with artemisinin on reticulocyte counts in malaria. The report by Cao et al. showed a rapidly reversible negative effect of artemisinin compared with quinine, on reticulocytosis, in (severe) malaria. However, the patient groups differed in the route of administration (intravenous quinine versus rectal and intramuscular artemisinin derivatives). Conversely, the results of the present study in children with uncomplicated malaria showed a typical, appropriate bone marrow response, and did not suggest a significant suppressive effect. The discrepancy between this and the available study on this subject is likely due to differential bone marrow response of individuals with uncomplicated versus severe malaria to erythropoiesis. It should also be mentioned that, the study by Cao et al. [7] only found a difference between the groups on day 5 of treatment (which is a day with expected marked reticulocytosis due to the clearance of parasites and/or as-yet uncorrected anaemia).

Several studies have shown a marked suppression of erythropoiesis during malaria [2,6]. This effect has been variously ascribed to dysregulation of the immune response to the infection [2,9-11]. However, in recent years an increasing focus on the direct toxic effect of haemozoin, formed as a residual product by the parasites [12,13], and mediated through down regulation of haematopoietic growth factors [14] is being made. Notably, the suppression of erythropoiesis is rapidly reversible as soon as parasites have been cleared from the circulation [5] and demonstrated by the reticulocyte count dynamics shown in this study (Figure 1). Conversely, it has been shown that low numbers of parasites in asymptomatic malaria, or sub-microscopic parasitaemia, could maintain dyserythropoiesis for prolonged periods [15,16], and the relatively low reticulocyte count on day 28 reverting to pre-illness levels, or sustained bone marrow depression.

The artemisinin derivatives are the most rapidly acting antimalarials known, with their antimalarial action partially mediated through an increase in the production of intracellular reactive oxygen intermediates [5]. The initially higher fractional reticulocyte change from baseline in the two ACT groups compared with the amodiaquine group, likely reflects the rapid parasite clearance expected in these groups while the higher overall fractional reticulocyte changes between baseline (acute illness) and the presumed steady state day 28 post treatment levels in the amodiaquine-based treatment groups likely reflects a more profound effect of these treatments, or a relatively slower parasite clearance or both. On the other hand, it is also possible that, any such effects of the artemisinin derivatives are not significant beyond early embryonic stages, since it has been shown that any potential effects of artemisinin derivatives,

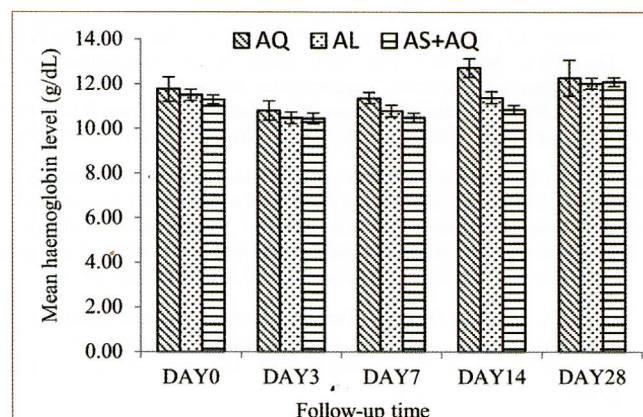


Figure 2: Mean haemoglobin levels (g/dL)

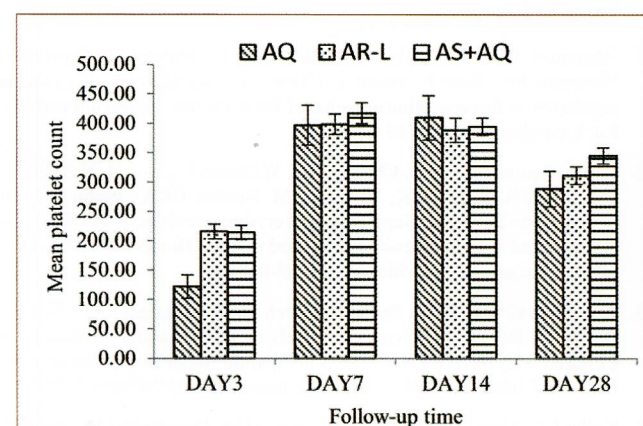


Figure 3: Mean platelet counts

Table 3: Fractional changes in selected reticulocytes between day 3 and day 28 of treatment in the children treated with AQ, AL or AS+AQ

Group	Mean difference (95% CI) [day 3 - day 28]	Proportional difference (per endpoint)	p value
AQ	7.37 (-13.59 - 28.34)	125.53%	0.46
AL	-0.43 (-3.41 - 3.55)	6.06%	0.78
AS+AQ	2.34 (-0.37 - 5.05)	45.92%	0.09

*AQ, Amodiaquine; AL, Artemether-lumefantrine; AS+AQ, Artesunate-amodiaquine; CI, confidence interval.

though significant in young embryos, caused only minor changes in reticulocyte counts in later foetal stages [3].

Conclusion

In conclusion, treatment of children with acute uncomplicated malaria showed an overall marginally lower reticulocyte count change dynamics in those treated with artesunate-amodiaquine or artemether-lumefantrine, compared with those treated with amodiaquine. However, the discrepancy between this study and the only available report on this subject call for additional studies, and post-marketing surveillance to confirm that any such changes are minimal and reversible, even in potential vulnerable patient sub-groups, is indicated.

DECLARATIONS

Ethical considerations

The study was approved by the Ethics and Protocol Review Committee of the University of Ghana Medical School (MS-Et/1-P.5.4/2009-2010).

Consent to publish

All authors agreed to content of the final paper.

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Competing Interests

No potential conflict of interest was reported by the authors.

Author contributions

The study was conceived and designed by GOA, BQG, MA, and JAL. The clinical work was done by GOA, BQG and JAL. The laboratory work was done by MMA, GOA, and AMS. The data was analysed by AMS and GOA. The manuscript was drafted by GOA and all authors contributed to the final draft.

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Availability of data

Data is available upon request to the corresponding author.

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