



SDI Review Form 1.6

Journal Name:	South Asian Journal of Parasitology
Manuscript Number:	Ms_SAJP_41651
Title of the Manuscript:	Efficacy of Artesunate-amodiaquine combination therapy against Plasmodium falciparum malaria in a forty-two day follow-up in the Ikata-Likoko area of Southwest Cameroon
Type of the Article	Original Research Article

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Introduction, "It has been reported to be efficacious, safe and well tolerated in several studies [4, 5, 8-12]": The reference citations should refer to only the studies performed in Cameroon or to a meta-analysis. Ref 8 was conducted in Congo. My bibliographic research shows that several important clinical studies on ACT have been conducted in Cameroon but have not been cited by the authors. There seem to be at least three research teams that have conducted therapeutic efficacy studies in Cameroon, including the groups of Basco, Mbacham/Leke, and Achidi. The authors should cite their works, including the following: Sagara et al. Malaria J 2009;8:63; Whegang Youdom et al. Malaria J 2010;9:56; Whegang Youdom et al. Malaria J 2012;11:147; Tahar et al. J Infect Dis 2014;210:1962-1971; Nji et al. Malaria J 2015;14:27. Among these studies, the authors should note in Discussion that the group of Achidi has conducted clinical studies in Mount Cameroon also. Materials and methods, 2.3 Study population, "Also, participants who were resident out of the study area... or lost to follow-up were excluded from the study": WHO protocol does NOT exclude patients who are lost to follow-up. Later in the Materials and methods section, the authors state, in section 2.8, that both per protocol (PP) and ITT analyses were done. In ITT population, ALL patients who were initially included in the study are included in analysis. This includes patients who were excluded (e.g. withdrawal of consent) and lost to follow-up. This should be made very clear, not only in the materials and methods section, but also in the Results section. Materials and methods, 2.7 "Instructions were given to participants.. treatment on days 2 and 3" and "They were contacted on phone at least once a day from day 1 to day 6...": The counting method is not clear. WHO considers the first day (patient inclusion) as day 0. Treatment is administered on days 0, 1, and 2. Since the authors used the WHO protocol, they should abide by the WHO methods. In section 2.7, the authors should state more clearly that (1) ASAQ administration was supervised on day 0 (1st dose) and unsupervised on days 1 (2nd dose) and 2 (3rd dose) and that (2) WHO requires a follow-up on days 2 and 3, with clinical assessment and microscopic examination of Giemsa-stained smears. Based on the authors' earlier statements (see Section 2.3 "the protocol for populations with moderate endemicity was applied as indicated in WHO, 2009"), readers are misled about the follow-up visits, which should be on day 1 (for supervised ASAQ treatment, 2nd dose, and clinical assessment), day 2 (for supervised ASAQ treatment, 3rd dose, clinical assessment, and blood examination), day 3 (for clinical assessment and blood examination), then on days 7, 14, 21, 28 (35 and 42). In section 3.4, the authors state that the mean Hb value was lowest on day zero. Please clarify the days in section 2.7. Materials and methods, 2.8 outcomes: In relation to my previous comments above, the readers should explain why "early treatment failure" cannot be evaluated in the modified WHO protocol that the authors used. Likewise, "higher parasite count of blood than the previous count which was not negative, as well as a positive sample that was negative during the previous reading were also considered as failures" is authors' own criterion which is not present in the WHO protocol. Taken together, my recommendation is to highlight the authors' modifications of the standard WHO protocol. Results, 3.1, "The distribution in terms of age, sex and locality was comparable in 136 participants": What are the authors comparing these values to? There is no comparison. This statement should be revised.	The authors very much appreciate comments of the reviewer. Reference 8 has been replaced by a meta-analysis by Whegang Youdom et al, 2017. Refs. 10 and 11 are meta-analysis. References 9, 12 and also 26 and 28 are studies carried out in Cameroon. 12 was done by both Mbacham and Achidi teams; Ref. 26 (Nji et al., 2015) by Mbacham's team while 28 was done by Achidi's team. With respect to the discussion, reference 28 by Apinjah et al., 2016 carried out in the mount Cameroon area by Achidi's team has been cited and findings compared with those of this study. Agreed. Participants who withdrew consent and those who were lost to follow-up were included in the analysis Agreed and reviewed as recommended by reviewer. However, these were mentioned as some of the limits of the study. Agreed. Days have been clarified in section 2.7 Agreed, reviewed as recommended by reviewer Agreed, statement has been revised. Really sorry about that. However, Figure one was sent as a separate attachment. Probably the editor didn't forward to you. Agreed. Table 2 has been reviewed. Populations of ITT and PP have been added. Agreed. Bars showing standard deviation of haemoglobin values have been added



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	Figure 1: The figure I received is empty. I cannot evaluate this figure. Table 2: This table is very confusing. It should be revised. If there were 97 ACPR, 4 LCF, 4 LPF, and 35 lost to follow-up or withdrawal, the total is 140. The text (section 3.2) says that 101 participants completed the follow-up and that there were 4 failures. The table shows 8 failures. Please clarify. The table should show the number of ITT and PP populations. Figure 3: Bars showing the standard deviation of hemoglobin values should be added. Table 3: Standard deviations should be provided for each mean values. The authors should explain in the table legend which values are being compared for the level of significance. Is it between day 0 values and day 7 values? Results, section 3.6: The authors should provide more information. Did these adverse effects require hospitalization or were they mild and transient? Discussion, "The ACPR of 96.0% on day 42 shows that it is effective against..." While the statement is true, it does not provide a pertinent information to readers. Many review papers have shown that ACT efficacy remains high (> 95%) in Africa. Instead of comparing their results with those of Liberia (Ref 19) and Uganda (Ref 27), the authors should cite other clinical studies on AS-AQ performed in Cameroon, including earlier studies done in Mount Cameroon area (see the works of E. Achidi). Discussion, "the efficacy might have reduced though the studies were not carried out in the same population" and "...due to a possible reduction in efficacy with time": It is difficult to assess efficacy with ITT population, especially when the rates of lost to follow-up and exclusion are high. The authors should provide more convincing evidence that ACT efficacy is on the decline in Cameroon or cite references to support their claim on reduced ACT efficacy. Discussion, "There was a general decrease in cases of fever..." to "...may have been due to other factors including other infections or level of parasitaemia". In all clinical studies on ACT and many other antimalarial drugs, body temperature decreases after a complete treatment. These three statements do not add any useful information. They should be deleted. Discussion, "The decrease in WBCs during follow-up could be due to neutropenia associated with hepatotoxicity..." This statement should be deleted. Two of the rare but serious adverse effects of amodiaquine are pancytopenia and hepatotoxicity. These two seem to be 'independent' side effects. Discussion, "...could be due to an attenuation effect of amodiaquine on artemisinin": This part of the statement is not clear. Please clarify. Discussion, "The observed general decrease in values of MCV..." to "...their sizes reduce and consequently the MCV values": This is speculative. These statements should be deleted unless the authors can cite references supporting their claim.	Standard deviations have been added for each mean value More information has been provided with regards to adverse events References 26 and 28 cited here are studies carried out in Cameroon with 28 carried out in the Mount Cameroon area by Achidi's team. It may be difficult to assess efficacy using the ITT population, but the information is quite complementary. The ITT results here have been compared to two other studies carried out in Cameroon (Nji et al. and Apinjoh et al.) True, body temperature reduces following treatment with AS-AQ, this statements were made to highlight cases of fever reported later during follow-up, given that fever was assessed during the study. Agreed. Sentence has been deleted The statements that follow explain this already. Firstly that, artemisinin favours spitting with increase in RBC; and that amodiaquin attenuates artemisinin activity as reported by Sowunmi et al, consequently spitting is disfavoured and so reduction in RBCs as observed in this study.
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Minor REVISION comments	List of abbreviations: The authors do not provide what each abbreviation stands for. Introduction: “Although there HAS been a scale-up...” Introduction: “Although these measures would reduce the morbidity and mortality...during preliminary studies (ADD REFERENCES HERE)” Introduction, “ACTs consist of two active metabolites”: The term “metabolite” is not appropriate. Introduction, Ref 4: The latest WHO Guidelines for the treatment of malaria (3rd edition, 2015) should be cited. Introduction, “...produce faster relieve...”: relief Introduction, “However, despite its efficacy, a decline in parasite clearance rates in ACT... (16-18)”: More appropriate studies should be cited here. Ref 17 is an in vitro work. It should be deleted or replaced by a clinical study. Introduction, “In Cameroon, Ali et al reported the development of resistant alleles...”: The parasites do not DEVELOP resistant alleles. Introduction, “Efficacy could be assessed either by the use of molecular markers...”: WHO recommends therapeutic efficacy studies as the gold standard. The term “in vivo assay” should be replaced by “therapeutic efficacy studies,” not only in this sentence, but elsewhere in the manuscript. Moreover, the references 20 and 21 are not appropriate here to support the authors’ statement. Introduction, “The response to treatment...may be of invaluable efficacy assessment..” The expression is awkward. This sentence should be rewritten. Introduction, “Specifically by assessing...”: This is not a complete sentence. Materials and methods: General readers would probably not know where Ikata-Likoko is. An earlier study conducted in this locality (Ref 22) does not provide a map. A map of Cameroon indicating its location would be helpful. Materials and methods, 2.5 Clinical assessment of participants: The manufacturer of the floor scale is not required. It can be deleted. Materials and methods, 2.6.3: A period (.) after “(URIT 3300 analyser).” Materials and methods, 2.9, GMPD: Please introduce in the text, when first used, the meaning of the abbreviations. Not all readers will know that the authors are referring to geometric mean parasite density. Materials and methods, 2.9, “P-values less than .5”: It should be 0.05 or 5%. See also section 3.4 “the values however increased on day 21... (P > .5)” Correct P values are used in section 3.5. Results, 3.1: The distribution...WAS comparable Results, 3.3: The statements “the mean temperature on day 0 was significantly higher than any other day...” should be deleted. All clinical studies have shown the same phenomenon, and this observation is an expected, obvious outcome.	The journal recommends that each abbreviation should be written in full upon first usage in text. This has been done including those that were omitted. Agreed. Corrected Preliminary studies here refer to visits we made on the field before this study. The statement has been revised for clarity. “Metabolite” has been replaced with “molecules” This study was carried out before the 3rd edition was published. Corrected Agreed. Reference 17 has been deleted Corrected Agreed and corrected as recommended Agreed. Reference 20 has been deleted. Reference 21 now 19 is a clinical study so authors think it should be maintained Sentence has been revised Sentence has been completed True, the reference does not provide a map but provides a detail geographical location of the study site Manufacturer's name has been deleted Period added GMPD has been written in full Agreed. P-values have corrected Sentence has been revised True, clinical studies have shown the phenomenon but it is necessary to report it here because it was assessed. Besides, we think it gives the assurance that AS-AQ still reduces fever in falciparum malaria.
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	Results, 3.4: The statement “The GMPD reduced to zero/μl on day 21...” should be deleted. The second statement “All participants remained negative...” is saying the same thing. What is MP? Discussion, Ref 29, “...due to the variability in pharmacokinetics profiles...”: Ref 29 is an in vitro work. It does not support the authors’ statement. Please replace or delete it. Discussion, “...which has been linked with differences in the level of drug response with outcome including incomplete cure and relapse”: Relapse occurs only with P. vivax and P. ovale malaria. The authors probably mean to say “recrudescence.” Ref 33: Please provide the article title.	Statement GMPD... has been deleted Agreed. Reference 29 has been deleted Agreed, we meant “recrudescence” Article title for reference 33 has been provided
<u>Optional/General</u> comments	The authors assessed the efficacy of artesunate-amodiaquine to treat uncomplicated falciparum malaria in Mount Cameroon area. The authors considerably modified the standard WHO protocol for evaluation of therapeutic efficacy. The most serious modification is the lack of PCR correction, required in the WHO protocol to distinguish between reinfection (which is not a treatment failure) and recrudescence. English needs many corrections. Because of time constraints requested by the editor, I did not note all English mistakes in the manuscript.	Agreed. This has been cited as a limit and justifications given for the modification of the protocol. However, authors believe that this work carries some interesting findings on AS-AQ efficacy.