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Titre de la thèse :

Méthodes d'analyse statistique pour données répétées dans les essais cliniques : intérêts et applications au paludisme

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Résumé

De nombreuses études cliniques ou interventions de lutte ont été faites ou sont en cours en Afrique pour la lutte contre le fléau du paludisme. Pour une lutte efficiente contre cette maladie, les essais cliniques, évaluant de nouvelles molécules ou stratégies, doivent réponde à des définitions plus proches de la réalité de terrain et les données doivent être analysées avec des méthodes statistiques appropriées. En zone d'endémie, le paludisme est une maladie récurrente. La répétition des crises palustres est donc une des cibles à atteindre lorsqu'il s'agit d'évaluer un traitement ou une stratégie. Or, la revue de littérature indique une application limitée des outils statistiques appropriés existants pour l'analyse des données récurrentes de paludisme. Au cours de cette thèse, nous avons travaillé sur une définition du critère de jugement principal prenant en compte la répétition des évènements ou des mesures, et mis en œuvre des méthodes statistiques appropriées pour l'analyse de ces données issues d'essais cliniques de traitements antipaludiques. Ces données concernaient les épisodes répétés de paludisme. Le patient recevait le même traitement à chaque épisode durant la durée de l'étude, afin d'identifier les traitements réduisant le nombre d'épisodes. Nous avons également étudié les mesures répétées d'hémoglobine lors du suivi de traitements antipaludiques en vue d'évaluer la tolérance ou sécurité des médicaments en regroupant les données individuelles de 13 essais cliniques.

Pour l'analyse du nombre d'épisodes de paludisme, la régression binomiale négative a été mise en œuvre. Pour modéliser la récurrence des épisodes de paludisme, quatre modèles ont été utilisés : i) Les équations d'estimation généralisées (GEE) utilisant la distribution de Poisson; et trois modèles qui sont une extension du modèle Cox: ii) le modèle de processus de comptage d'Andersen-Gill (AG-CP), iii) le modèle de processus de comptage de Prentice-Williams-Peterson (PWP-CP); et iv) le modèle de Fragilité partagée de distribution gamma. Pour l'analyse de sécurité, c'est-à-dire l'évaluation de l'impact de traitements antipaludiques

sur le taux d'hémoglobine ou la survenue de l'anémie, les modèles linéaires et latents généralisés mixtes (« GLLAMM : generalized linear and latent mixed models ») ont été mis en œuvre.

Ce travail a identifié un critère de jugement dans le cadre des essais clinique pour le paludisme, critère plus conforme à une réalité de terrain et de santé publique. Nous avons montré comment appliquer correctement les outils statistiques existants dans l'analyse de ces données. Les perspectives de ce travail demeurent dans l'élaboration de guides de bonnes pratiques de références pour la méthodologie de préparation et d'analyse des données de paludisme récurrent, la création d'un entrepôt de données issues d'essais cliniques facilitant l'analyse de données regroupées.

Mots clés: Paludisme, Essais cliniques, Traitements, Evènements récurrents, Extension du modèle Cox, Modèle de fragilité, Equations d'Estimation Généralisés (GEE), Modèle à variable latente (GLLAMM), Sécurité, Données regroupées ou agrégées.

Abstract

Numerous clinical studies or control interventions were done or are ongoing in Africa for malaria control. For an efficient control of this disease, the clinical trials evaluating new molecules or strategies, should meet closer to the reality of field endpoints and the data should be analyzed using appropriate statistical methods. In endemic areas, malaria is a recurrent disease. The repetition of the malaria crisis is one of targets when it comes to evaluate a treatment or a strategy. However, the literature review indicates a limited application of appropriate statistical tools for the analysis of recurrent malaria data. In this thesis, we have worked on a definition of the main judgment criterion taking into account repetition of events or measures, and implementation of statistical methods appropriate for the analysis of these data from clinical trials of antimalarial treatments. These data were the repeated episodes of malaria. The patient received the same treatment to each episode during the duration of the study, in order to identify the treatments associated with less malaria episodes. We have also studied the repeated measurements of hemoglobin during malaria treatments follow-up in order to assess the tolerance or safety of the study drugs by pooling individual data from 13 clinical trials.

For the analysis of the number of malaria episodes, the negative binomial regression has been implemented. To model the recurrence of malaria episodes, four models were used: i) the generalized estimating equations (GEE) using the Poisson distribution; and three models that are an extension of the Cox model: ii) Andersen-Gill counting process (AG-CP), iii) Prentice-Williams-Peterson counting process (PWP-CP); and (iv) the shared gamma frailty model. For the safety analysis, i.e. the assessment of the impact of malaria treatment on hemoglobin levels or the onset of anemia, the generalized linear and latent mixed models (GLLAMM) has been implemented.

This work identified a judgment criterion in the context of malaria clinical studies fitting field reality and public health. We have shown how to properly apply the existing statistical tools in the analysis of these data. The prospects of this work remain in the development of guides on good practices of references to the methodology for the preparation and analysis of recurrent malaria data, the establishment of data storage network for malaria clinical trials facilitating the analysis of pooled individual data from clinical trials.

Key words: Malaria, Clinical trials, Treatments, Recurrent events, Extended Cox model, Frailty model, Generalized estimating equation (GEE), Generalized linear and latent mixed model (GLLAMM), Safety, Pooled data.

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2. **Sagara I,** Giorgi R, Doumbo OK, Piarroux R, Gaudart J. Modelling recurrent events: comparison of statistical models with continuous and discontinuous risk intervals on recurrent malaria episodes data. Malar J. 2014 Jul 29;13:293. doi: 10.1186/1475-2875-13-293.

3. **Sagara I,** Piarroux R, Djimde A, Giorgi R, Kayentao K, Doumbo OK, Gaudart J. Delayed anemia assessment in patients treated with oral artemisinin derivatives for uncomplicated malaria: a pooled analysis of clinical trials data from Mali. Malar J. 2014 Sep 12;13:358. doi: 10.1186/1475-2875-13-358.

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2. Communication orale au congrès International de la Statistique Appliquée pour le Développement en Afrique (SADA): **Issaka Sagara**, Ogobara K Doumbo, Roch Giorgi et Jean Gaudart. Modeling recurrent events: comparison of statistical models with continuous and discontinuous risk intervals on repeated malaria episodes data. SADA 2013; 5-8 March 2013 Cotonou, Benin (http://sada2013.sciencesconf.org)
3. Communication orale (MO.27) au *« Seventh EDCTP Forum, Berlin, Germany, 30 June-02 July 2014 »*: **Issaka Sagara**, Ogobara K Doumbo, Abdoulaye Djimdé, Alassane Dicko, Roch Giorgi, Jean Gaudart. Modelling recurrent events: comparison of statistical models with continuous and discontinuous risk intervals on repeated malaria episodes data.
4. Communication poster acceptée au congrès International du *« 63rd Annual Meeting of American Society of Tropical Medicine and Hygiene, New Orleans, USA, 2-6 November 2014 »* : **Issaka Sagara**, Renaud Piarroux, Abdoulaye Djimde, Roch Giorgi, Kassoum Kayentao, Ogobara K Doumbo, Jean Gaudart. Delayed anemia assessment in patients treated with oral artemisinin derivatives for uncomplicated malaria: a pooled analysis of clinical trials data from Mali. Abstract#1491.

Liste des sigles et abréviations

AG-CP	Andersen-Gill counting process
AL	Artéméther-luméfantrine
AQ	Amodiaquine
AS+AQ	Artésunate/amodiaquine
AS-MEF	Artésunate/ Méfloquine
ASPYR	Artésunate-pyronaridine
AS-SMP	Artésunate/sulfaméthoxypyrazine-pyriméthamine
AS+SP	Artésunate/sulfadoxine-pyriméthamine
CAT	Combinaisons Thérapeutiques à base d'artémisinine
CQ	Chloroquine
EDCTP	European & Developing Countries Clinical Trials Partnership
ES	Erreurs standards
GEE	Les équations d'estimation généralisées
GLLAMM	Generalized linear and latent mixed model
MRTC	Malaria Research and Training Center
OMS	Organisation Mondiale de la Santé
PWP-CP	Prentice-Williams-Peterson counting process
RR	Risque relatif
WANECAM	West African network for clinical trials of antimalarial drugs
WLW	Wei-Lin-Weissfeld

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I. Introduction

Afin d'apprécier de façon optimale l'efficacité et la sécurité de schémas thérapeutiques ou de stratégies de lutte contre le paludisme, la définition de critère de jugements proche de la réalité de terrain et l'application d'analyses statistiques appropriées s'impose. Le paludisme, avec 216 millions de cas cliniques en 2012, dont 91% en Afrique [1], est un fléau pour le continent Africain. Le nombre de décès est estimé à 627 000 décès dont 89% en Afrique Subsaharienne [1].

Dans les essais cliniques de médicaments ou de vaccins, l'intérêt porte sur l'efficacité et la sécurité ou tolérance du produit. Ce type d'étude peut être une étude de phase 3 de supériorité, ou de non infériorité, aboutissant à la mise sur le marché d'un produit antipaludique si son efficacité, ou sa non infériorité, est prouvée.

Dans les schémas classiques des essais thérapeutiques de paludisme, le patient reçoit, généralement après randomisation, un des traitements antipaludiques, et, est suivi lors des visites régulières (ou imprévues) intermittentes pendant une durée de 28 ou 42 ou 63 jours, conforment au guide d'évaluation de traitement antipaludique de l'Organisation Mondiale de la Santé [2]. Lors de ces visites intermittentes, l'efficacité du produit après son administration est mesurée par l'évaluation clinique et parasitologique et généralement, seul le premier épisode est analysé. L'analyse couramment faite reposant sur ce type de données utilise l'estimateur de Kaplan-Meier [3] ou même est restreinte à une comparaison de pourcentages d'efficacité [4]. L'estimateur de Kaplan-Meier tient compte du temps de participation à l'étude de chacun, et des censures à droite, mais ne permet que l'analyse d'un seul évènement. Nous avons conduit au Mali plusieurs études cliniques de phase 3, analysé (par l'estimateur de Kaplan-Meier ou comparaison de pourcentages) et publié des données d'efficacité de plusieurs traitements antipaludiques [5-8].

En zone d'endémie palustre, les patients ont généralement plus d'un épisode clinique, mais les différents épisodes ne sont que très rarement étudiés dans les essais cliniques. Il est cependant possible de définir l'efficacité non pas seulement avec le premier épisode, mais, soit en mesurant le nombre d'épisode, ce qui correspond à un critère de jugement de "santé publique" (pouvant avoir une interprétation en terme de dynamique épidémique, de l'effet préventif post-traitement et de coût) [9], soit en mesurant la récurrence des épisodes à différents délais (donnant une interprétation possible quand à la dynamique de l'infection).

Une analyse appropriée de ces données répétées ou récurrentes de paludisme tiendra compte du fait que les événements récurrents chez la même personne sont non indépendants. Par ailleurs, le traitement curatif de chaque épisode implique une période de "washout" dépendant de la demi-vie du traitement. Il est donc nécessaire d'en tenir compte avec des modèles de risque à intervalles discontinus [10] pour obtenir des estimations non biaisées des taux d'incidence de l'événement ou maladie. La période pendant laquelle le sujet n'est pas à risque de la maladie devrait être exclue du temps de risque de cette maladie. Le sujet n'est pas à risque d'un autre événement pendant que le précédent évènement est en cours ou que le traitement est en cours ou avant la fin de la période estimée de l'effet préventif du traitement antérieur.

Outre les études d'efficacité, l'analyse appropriée de la sécurité ou de la tolérance d'un produit d'étude clinique s'impose conformément à la recommandation internationale [11]. Cette évaluation est capitale et l'analyse des données de sécurité ou tolérance peut conduire à l'arrêt du développement clinique du produit ou du retrait d'un produit déjà commercialisé. Cependant, dans certains cas, les essais cliniques individuels ne sont pas de taille suffisante pour mettre en évidence un effet indésirable rare ou inattendu, ou mal identifié. Dans ce contexte, l'agrégation des données de plusieurs essais thérapeutiques peut permettre d'augmenter la puissance d'analyse, avant des études en population (de type phase IV).

L’objectif de ce travail est la mise en œuvre des méthodes statistiques adaptées existantes pour répondre au besoin d’analyse des données d’essais cliniques de différents traitements antipaludiques pour évaluer soit leur intérêt en terme de réduction de nombre d’épisodes, soit leur sécurité clinique, en occurrence leur effet sur l’hémoglobine. Notre approche vise à répondre à des questions non adressées lors d'essais cliniques classiques d'antipaludiques, pourtant plus proches de la réalité de terrain. Nous avons ainsi adapté le critère de jugement et utilisé, et discuté, les méthodologies statistiques avancées appropriées.

Le manuscrit est présenté en trois parties:

Dans la première partie, nous avons défini comme critère de jugement en santé publique le nombre d'accès palustres par bras de traitement, et utilisé de méthode statistique adaptée.

Dans la deuxième partie, nous avons comparé les principales méthodes statistiques pour analyser les évènements récurrents de paludisme.

Dans la troisième partie, nous avons étudié l'anémie post-thérapeutique, en agrégeant les données issues de différents essais cliniques de traitements antipaludiques, et mis en œuvre des méthodes statistiques adaptées.

II. Analyse de données pour l'évaluation d'efficacité thérapeutique, et l'intérêt en santé publique du choix d'un antipaludique

Lors des essais cliniques de traitements antipaludiques, l'efficacité est définie par la disparition de la symptomatologie et de la parasitémie après l'administration d'un traitement antipaludique et qui est maintenue jusqu'à une durée déterminée (28 ou 42 ou 63 jours en fonction du médicament utilisé) [2]. Cependant, en terme de santé publique, il est plus intéressant d'évaluer le nombre d'accès palustres plutôt que de restreindre l'analyse au seul accès palustre. Ainsi, il est possible d'en tirer des renseignements quant à la dynamique de l'épidémie, et au coût de la prise en charge du fait de la différence de nombre d'épisodes entre les traitements.

Pour répondre à la question d'efficacité thérapeutique de différentes combinaisons thérapeutiques à base d'artémisinine (CTA), nous avons analysé l'efficacité d'un produit vis-à-vis du paludisme pour un épisode isolé donné de paludisme qui avait été suivi et évalué sur 28 jours conformément au guide de l'OMS [2]. Nous avons aussi analysé le nombre d'accès palustres par bras de traitement pour identifier les traitements de choix qui sont associés à moins d'épisodes de paludisme pendant la période d'étude, donc d'intérêt en santé publique. L'étude avait été conduite de Juillet 2005 à Juillet 2007 dans le site de recherche du MRTC à Bougoula-Hameau, un quartier périphérique de Sikasso, dans la région sud du Mali. L'inclusion des patients (n=780) a été faite de manière progressive entre Juillet 2005 et Octobre 2006 à l'occasion d'un premier accès palustre. Les médicaments concernés étaient : l'artéméther-luméfantrine (AL), l'artésunate/amodiaquine (AS+AQ) et l'artésunate/sulfadoxine-pyriméthamine (AS+SP). Pendant toute la durée de l'étude, à chaque épisode de paludisme, le patient recevait le même traitement antipaludique qu'il avait reçu au début de l'étude à l'enrôlement et est suivi et évalué sur 28 jours conformément au guide de l'efficacité thérapeutique de l'OMS [2]. Le taux d'efficacité thérapeutique par bras de

traitement « Per protocole » et en « Intention de traiter » et les tests de comparaison de taux d'efficacité sont donnés dans le Tableau 3 de nos travaux publiés en 2012 dans « *American Journal of Tropical Medicine and Hygiene* » dont l'article est joint ci-dessous. Ce tableau indique, un taux d'efficacité (efficacité corrigée par la biologie moléculaire en excluant les cas de nouvelle infection lors de suivi de 28 jours) élevé (>98%) et comparable entre les trois bras de traitement pour l'analyse « Per protocole », pendant que le taux d'efficacité par « Intention de traiter » ainsi que le taux de réinfection différaient significativement entre les trois bras de traitement.

Pour déterminer lequel des trois traitements antipaludiques avait un intérêt en terme de santé publique durant la période d'étude, nous avons analysé les données avec une méthode statistique d'analyse de données de comptages des épisodes. L'analyse multivariée a utilisé le modèle de régression binomiale négative. Les risques relatifs (RR) étaient estimés de cette équation de régression ainsi que leurs intervalles de confiance. Les résultats de cette analyse, comme indiqués dans le Tableau 2, ont montré que l'AS+SP, suivi par l'AS+AQ étaient significativement associés à moins d'épisodes de paludisme (effet protecteur) par rapport à l'AL ; RR=0.80 (0.72–0.89) et RR=0.84 (0. 75–0.93) respectivement.

Article 1: « Repeated Artemisinin-Based Combination Therapies in a Malaria Hyperendemic Area of Mali: Efficacy, Safety, and Public Health Impact »

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Repeated Artemisinin-Based Combination Therapies in a Malaria Hyperendemic Area of Mali: Efficacy, Safety, and Public Health Impact

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Abstract. Artemisinin-based combination therapies (ACTs) are the first-line treatment of uncomplicated malaria. The public health benefit and safety of repeated administration of a given ACT are poorly studied. We conducted a randomized trial comparing artemether-lumefantrine, artesunate plus amodiaquine (AS+AQ) and artesunate plus sulfadoxine-pyrimethamine (AS+SP) in patients 6 months of age and older with uncomplicated malaria in Mali from July 2005 to July 2007. The patient received the same initial treatment of each subsequent uncomplicated malaria episode except for treatment failures where quinine was used. Overall, 780 patients were included. Patients in the AS+AQ and AS+SP arms had significantly less risk of having malaria episodes; risk ratio (RR) = 0.84 (P = 0.002) and RR = 0.80 (P = 0.001), respectively. The treatment efficacy was similar and above 95% in all arms. Although all drugs were highly efficacious and well tolerated, AS+AQ and AS+SP were associated with less episodes of malaria.

INTRODUCTION

According to the World health Organization (WHO) 2010 report, in the 106 malaria-endemic countries, 225 million cases of malaria led to an estimate of 781,000 deaths in 2009.[1] Once a person develops malaria, the only means of reducing suffering and preventing death is by diagnosing and treating the disease.[2] To counter the threat of resistance of *Plasmodium falciparum* to monotherapies, and to improve treatment outcome, WHO recommends that artemisinin-based combination therapies (ACTs) be used for the treatment of uncomplicated *P. falciparum* malaria.[2]

The choice of the ACTs in sub-Saharan African countries was mostly based on the efficacy and safety data of a single malaria episode treatment that does not take into account the long-term impact on malaria incidence and safety, which may have a given ACT when used over and over to treat consecutive malaria episodes.

In addition, as the first generation ACTs are being rolled out, studies show that they may rapidly increase the prevalence of molecular markers associated with resistance to the partner drugs, i.e., lumefantrine, amodiaquine, or sulfadoxine-pyrimethamine.[3–7]

Although data are available for the use of ACTs during single-episode treatment,[8–12] there are limited data on safety and public health benefits in terms of overall incidence of clinical episodes during the use of the same ACT during repeated malaria episodes treatment.[13–17]

To address these gaps this study aimed to evaluate the public health impact in terms of incidence of clinical episodes and the efficacy and safety of artemether-lumefantrine (AL), artesunate plus amodiaquine (AS+AQ), and artesunate plus sulfadoxine-pyrimethamine (AS+SP) when used repeatedly for consecutive clinical malaria episodes during as long as 24 months.

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MATERIALS AND METHODS

Study site. The study was conducted from July 2005 to July 2007 in Bougoula-Hameau, a peri-urban village of ~5,000 inhabitants located in the South of Mali. *Plasmodium falciparum* is hyperendemic with seasonal peaks that occur during the rainy season (June–November).

Study design and procedures. This was an open-label, randomized clinical trial comparing three different ACTs: AL, AS+AQ, and AS+SP.

When malaria was suspected finger-prick blood was taken for thick blood films. After ensuring that inclusion criteria are met, a written informed consent was obtained. Patients were included if they were at least 6 months of age, weighed ≥ 5 kg, resided in the study village, were able to receive oral treatment, had an axillary temperature ≥ 37.5°C, and had *Plasmodium* sp. infection with a parasite density between 2,000 and 200,000 asexual forms per microliter of blood for the first episode. The first subject was enrolled in July 2005 and the enrollment completed with the last subject enrolled 30 October 2006, although the last malaria episode follow-up ended in July 2007. Subjects with the following characteristics were not included in the study: the presence of severe or complicated malaria[18]; a severe concomitant pathology or one that needed a medical follow-up incompatible with the study, an allergy to one of the drugs involved in this study, and a pregnancy.

Eligible subjects were randomized to receive either AL or AS+AQ or AS+SP, and followed up for 28 days after the treatment initiation with one of the three ACTs, according to the 2003 WHO[19] treatment assessment guideline. Block-randomization was used to allocate the patients to the three treatment arms. Treatment assignments were through sequentially numbered opaque envelops that concealed the actual treatment to which the patient was randomized.

Once subjects were assigned to a given group, in the event of subsequent uncomplicated malaria episodes defined as the presence of malaria signs or symptoms plus *Plasmodium* sp. infection (a positive blood smear with parasite density < 200,000 parasites/μL), they were re-treated with that same treatment regimen (except in case of parasite recurrence cases occurring within each 28-day treatment follow-up

period where quinine was used). Patients were closely followed both clinically and biologically to record any adverse event. Patients with > 200,000 parasites/μL or who had treatment failure during the 28-day follow-up period or who developed severe malaria were treated with quinine and/or hospitalized if necessary. The study team was present 24 hours a day, 7 days a week at the study site during the study period.

Enrolled patients were asked to return for clinical and/or biological follow-up on Days 1, 2, 3, 7, 14, 21, 28, or on days of recurrent illness. Patients or guardians were asked about any medicine consumption outside the research facility since the last clinic visit. At each visit, a physical examination, including axillary temperature, was performed and blood was taken for thick smears. Patients were asked to return to the clinic any time if they became ill.

Thick blood films and blood onto filter paper samples were made on Days 0, 2, 3, 7, 14, 21, 28, and on any days of recurrent illness. Parasitemia were measured by counting the number of asexual parasites against 300 leukocytes on the thick blood film, and then reported to a microliter of blood based on a count of 7,500 leukocytes/μL.[20] If gametocytes were seen, a gametocyte count was performed against 1,000 leukocytes. Although this was an open-label study, all malaria slides were read in a blinded fashion by the technicians, because the parasitological result was the key element in the determination of the primary endpoint.

Blood blotted onto filter-paper samples were obtained for molecular analyses of drug resistance markers and to distinguish recrudescent from re-infections.

Hematology (hemogramme) and biochemistry (creatinemia and alanine aminotransferase) analysis were performed on Day 0, 7, and also on any other day if deemed clinically necessary to assess the biological toxicity of the study drugs. Normal values were based on laboratory parameters obtained from Malian population (our unpublished data). The values outside the normal ranges were considered abnormal and graded.

MSP1, MSP2, and CA1 polymorphisms were analyzed using standard methods with polymerase chain reaction (PCR) to discriminate true recrudescent parasites from new infections.[7]

Severe malaria cases and parasite recurrence cases occurring within each 28-day treatment follow-up period were treated with quinine (8 bmg/kg three times daily for 7 days) and re-enrolled (after complete remission) for the subsequent uncomplicated malaria (presence of malaria signs or symptoms plus *Plasmodium* sp. infection, which is defined as a positive blood smear with parasite density < 200, 000 parasites/μL) with the same study treatment arm. Quinine was chosen because it was the second-line drug for malaria treatment.

The efficacy data analysis was based on the WHO 2003, 28-day treatment efficacy assessment guideline[19]; the efficacy was determined in each subject after an uncomplicated malaria episode where the subject received the same ACT as during the initial randomization study arm. Treatment outcomes were classified as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), and adequate clinical and parasitological response (ACPR).

All study medicines were administrated orally by designated study personnel at the health center. The AS+AQ was Arsucam coblister (AS 50 mg/AQ 153 mg; Sanofi-Aventis, Paris, France), given once daily for 3 days to the following scheme: for patients < 10 kg, 1/2 tablet AS + 1/2 tablet AQ; 10–20 kg, 1 tablet AS+ 1 tablet AQ; 21–40 kg, 2 tablets AS+ 2 tablets AQ; > 40 kg, 4 tablets AS+ 4 tablets AQ.

The AS+SP was Arsumax (AS 50 mg; Sanofi-Aventis, Paris, France) + sulfadoxine–pyrimethamine (S _ 500 mg/P _ 25 mg, Fansidar Roche, Burlington, NC), given once daily for 3 days to the following scheme: for patients ≤ 10 kg, 1/2 tablet AS+ 1/2 tablet SP; 11–20 kg, 1 tablet AS+ 1 tablet SP; 21–40 kg, 2 tablets AS+ 2 tablets SP; > 40 kg, 4 tablets AS+ 3 tablets SP. The SP is given only the first day, whereas AS is given over 3 days.

The AL was Coartem (Novartis Pharma AG, Basel, Switzerland), given twice daily for 3 days to the following scheme: for patients < 15 kg, 1 tablet; 15–24 kg, 2 tablets; 25–34 kg, 3 tablets; ≥ 35 kg, 4 tablets.

Ethics. The study protocol was reviewed and approved by the Ethical Committee of the Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Mali. Community permission was obtained before the start of the study as described by Diallo and others.[21] Individual, written, informed consent was obtained from each patient (if adult) or parent or guardian of each child before enrolment.

Sample size. Calculation of sample size was based on the assumptions that patients experienced an average of two clinical episodes per person per year if treated by AL and

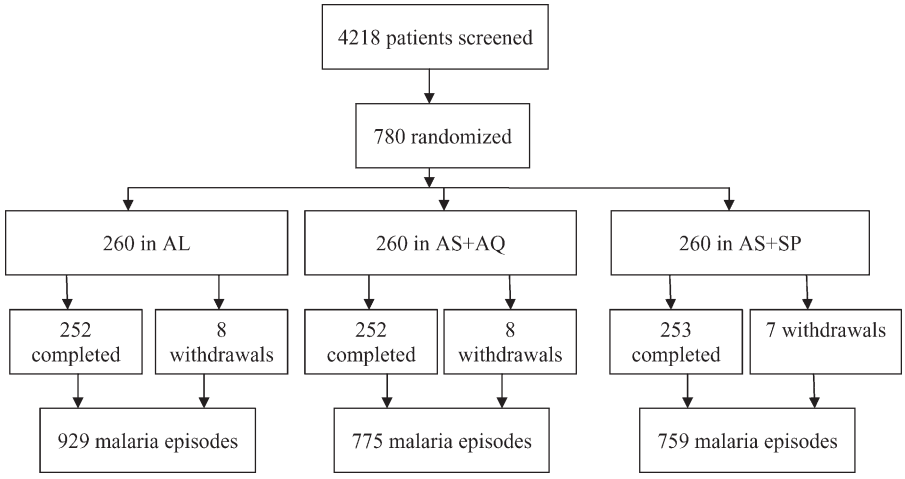

FIGURE 1. Study trial profile.

TABLE 1

Baseline characteristics per treatment group and overall at enrollment*

Characteristics	Regimen (AS+AQ) (*N* = 260)	Regimen (AS+SP) (*N* = 260)	Regimen (AL) (*N* = 260)	*P* value
Age (years)				
Mean ± SD	5.37 ± 6.19	5.23 ± 4.1	5.94 ± 6.8	0.51
Median (min, max)	4.1 (0.5,68.6)	4.3(0.5,30.5)	4.3(0.5,46.6)	
Age category n (%)				0.72
< 5	155 (59.6)	147 (56.5)	146 (56.2)	
5–9	78 (30.0)	85 (32.69)	88 (33.9)	
10–14	18 (6.9)	21 (8.1)	14 (5.4)	
≥ 15	9 (3.5)	7 (2.7)	12 (4.6)	
Gender – n (%)				
Female	142 (54.6)	129 (49.6)	130 (50.0)	0.45
P. falciparum (/μL)				
Median (Q1,Q3)	21,550 (10,375, 45,150)	23,225 (9,990, 44,190)	21,925 (10,440, 42,050)	0.99
Gametocyte– n (%)	16 (6.2)	18 (6.9) 28 (10.8)	0.11	

*n = frequency; Q1 = 25th percentile; Q3 = 75th percentile; Min = minimum; Max = maximum; AL = artemether-lumefantrine; AS+AQ = artesunate plus amodiaquine; AS+SP = artesunate plus sulfadoxine-pyrimethamine.

living in the study areas and that treatment with AS+AQ or AS+SP would reduce this value by 35%, which is 1.3 clinical episodes per person per year. With an error risk alpha set at 0.05, a power of 90%, 174 subjects were needed in each arm. If one takes into account loss of follow-up and data attrition, 260 subjects were needed in each arm (total sample of 780).

Data management and statistical procedures. Data were double entered and validated using Microsoft Access (Microsoft, Redmond, WA) and then analyzed using Stata software version 10.0 (StataCorp., College Station, TX).

For univariate analysis, χ^2 or Fisher's test if appropriate was performed to compare categorical variables. Normally distributed continuous variables were compared with analysis of variance. Nonparametric tests were used when appropriate.

Parasite recurrence occurring within each 28-day treatment follow-up was counted as a new malaria episode because it was associated with full malaria rescue treatment regimen. However, following 14 days after each malaria rescue treatment with quinine, the subject was not considered at risk of malaria, therefore that time was deducted in the subject's total length of time. Negative binomial regression analysis adjusted for repeated events for the same subject was used to estimate relative risk between study drugs.

The incidence rate is defined as number of malaria episodes in each study arm divided by the time length in days of each participant (person-time) and then expressed in a year (by dividing by 365.25 days).

Efficacy analysis concerned all combined malaria episodes from each participant. An intention-to-treat (ITT) analysis for drug efficacy was based on all subjects randomized to their respective treatment arm.

A per-protocol (PP) analysis for drug efficacy was also performed and excluded missing (lost to follow-up, withdrawal) data and major protocol violation cases.

For safety data, all subjects who received at least one dose starting from their first episode of malaria were included into the safety analysis.

RESULTS

Study trial profile and baseline characteristics. The trial profile is summarized in Figure 1. A total of 4,218 patients attended the clinic with malaria symptoms, 780 subjects who met the enrollment criteria were included in the study; 260 patients per treatment arm. A total of 2,463 malaria episodes were documented during the study, although 23 participants did not complete the study (Figure 1). The reasons for not completing the study were: travel (2 in the AL arm, 4 in the AS+AQ arm, and 3 in the AS+SP arm); consent withdrawal (4 in the AL arm, 1 in the AS+AQ arm, and 1in the AS+SP arm); treatment allocation errors that were not excluded from the study but adjusted into the analysis (1 subject received AS+AQ instead of AS+SP the original allocated treatment arm, 1 subject received AS+SP instead of AS+AQ the originally allocated treatment arm, 1 subject received AS+SP for the second episode instead of AL the originally allocated treatment arm); 2 cases of death in AS+SP arm;1 case in the AL arm received chloroquine treatment at home during the first malaria episode treatment; 1 case in the AS+AQ arm

TABLE 2

Incidence rates and RR per treatment arm and per age (in year) category*

Factors	Numbers of episodes (years at risk)	Incidence rate (95% CI)	Adjusted* RR (95% CI)	*P* value
AL	923 (341.42)	2.70 (2.53–2.88)	1	
AS+AQ	768 (340.59)	2.25 (2.12–2.44)	0.84 (0. 75–0.93)	0.001
AS+SP	756 (346.99)	2.18 (2.06–2.38)	0.80 (0.72–0.89)	< 0.001
Age ≥ 15	40 (41.07)	0.97 (0.70–1.33)	1	
Age 10–14	108 (71.44)	1.51 (1.24–1.83)	1.59 (1.07–2.34)	0.020
Age 5–9	779 (337.49)	2.31 (2.15–2.48)	2.41 (1.72–3.38)	< 0.001
Age < 5	1520 (579.0)	2.63 (2.49–2.76)	2.74 (1.96–3.82)	< 0.001

*Adjusted for age.
RR = risk ratio; AL = artemether-lumefantrine; AS+AQ = artesunate plus amodiaquine; S+SP = artesunate plus sulfadoxine-pyrimethamine; CI = confidence interval.

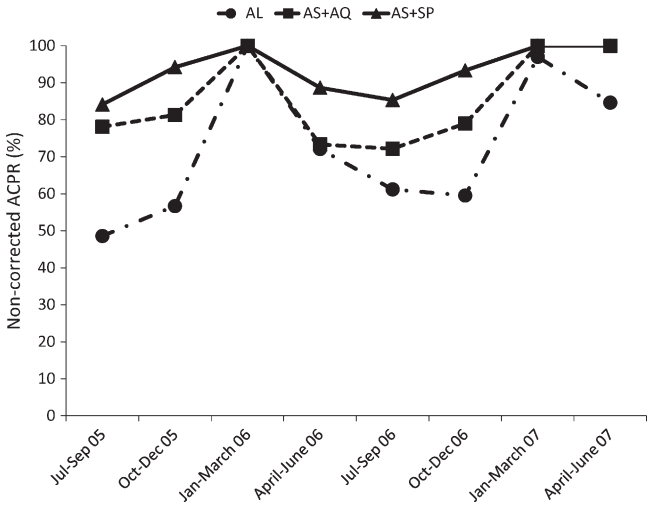

FIGURE 2. Per protocol non-polymerase chain reaction (PCR) corrected adequate clinical and parasitological responses (ACPRs) per study arm, per season, and per year. Non-PCR corrected ACPRs per study arm, per season, and per year. The numbers of patients (n/*N*) with non-corrected ACPRs were 51/105, 82/105, and 90/107, respectively, for AL, AS+AQ, and AS+SP in July–September 2005, 68/120, 87/107, and 114/121 in October–December 2005; 29/29, 25/25, and 22/22 in January–March 2006; 31/43, 33/45, and 47/53 in April–June 2006; 115/188, 127/176, and 163/191 in July–September 2006; 75/126, 94/119, and 140/150 in October–December 2006; 32/33, 24/24, and 16/16 in January–March 2007; 11/13, 13/13, and 12/12 in April–June 2007.

received quinine treatment at home during the second malaria episode treatment; and 1 case in the AS+AQ arm developed severe anemia during its first malaria episode treatment that required blood transfusion at the hospital.

At enrollment (Table 1), the mean age, sex ratio, the median parasitemia of *P. falciparum*, and the prevalence of gametocyte carriage were all similar in all three treatment arms. Only two cases of *Plasmodium ovale* and no case of *Plasmodium malariae* monospecific infection was detected, whereas 8% (N = 780) of *P. falciparum* + *P. malariae* and 0.8% (N = 780) of *P. falciparum* + *P. ovale* mixed infection were found (data not shown).

ACT and malaria incidence rate. Table 2 shows that the malaria incidence rate per person and per year was 2.70 (95% confidence interval [CI]: 2.53–2.88), 2.25 (95% CI: 2.12–2.44), and 2.18 (95% CI: 2.06–2.38) respectively in AL, AS+AQ, and AS+SP arms.

In multivariate modeling (Table 2), patients in the AS+AQ and AS+SP arms had significantly less risk of having malaria episodes than patients in the AL arm; risk ratio [RR] = 0.84 (P = 0.002) and RR = 0.80 (P = 0.001), respectively. Therefore, compared with AL the risk of malaria incidence reduction was 16% and 20% for AS+AQ and AS+SP, respectively. Using the age group ≥ 15 years of age as reference (Table 2), patients in the age group 10–14 years, the age group 5–9 years, and the age group < 5 years had significantly more risk of having malaria episodes; RR = 1.60 (P = 0.002), RR = 2.44 (P = 0.001), and RR = 2.76 (P = 0.001), respectively.

ACT efficacy on uncomplicated malaria. Non-PCR-corrected ACPR was significantly different between the study arms (62.0% (N = 665), 78.5% (N = 619), and 89.1% (N = 678) in AL, AS+AQ, and AS+SP arms, respectively, P < 0.001).

Figure 2 shows that there was a significant difference in the non-PCR-corrected ACPRs between the treatment arms according to the season. The lowest ACPRs were documented during rainy seasons when malaria transmission is highest (July–September followed by October–December). This seasonal variation in the efficacy of ACTs was documented for two consecutive years.

Using the ITT analysis (Table 3), the PCR-corrected ACPR were high and similar for all study arms 94.4%, 95.8%, and 97.9% in the AL, AS+AQ, and AS+SP arm, respectively. The difference of efficacy between any two arms treatment is < 5%.

Using the PP analysis (Table 3), the PCR-corrected ACPR were also high for all study arms; 99.1%, 98.7%, and 99.6% respectively in the AL, AS+AQ, and AS+SP arm.

Safety and tolerability. The adverse events with the highest incidence was mild vomiting, which was higher in AS+AQ and AS+SP than in the AL arm (P < 0.001), followed by abdominal pain and headaches (Table 4), which was similar in the three arms. One subject who had vomiting in the AS+AQ arm discontinued the oral treatment and parenteral quinine was provided because of the repeated drug dose vomiting. The frequency of the other adverse events was similar in the three arms. The incidence of laboratory adverse events was similar in the three treatments arms except for the creatinemia which significantly different; 5.09%, 6.33% and 15.19% in the AL, AS+AQ, and AS+SP arm respectively, P < 0.001.

Serious adverse events (SAEs) were documented and included 2 severe cases of anemia (1 in AL arm, 1 in AS+AQ arm); 2 cases of seizure (1 in AL arm, 1 in AS+AQ arm); 1 case of severe malaria (in AL arm); 1 case of coma caused by hypoglycemia (in AS+SP); 1 case of presumed pneumopathy

TABLE 3
Efficacy evaluation on Day 28 after PCR correction* ITT and PP analysis

	AL (N = 665)	AS+AQ (N = 619)	AS+SP (N = 678)	P value
Possible failure* – n (%)	31 (4.7)	18 (2.9)	11 (1.6)	0.005
ETF† – n (%)	0 (0)	1 (0.2)	0 (0)	0.32
LCF† – n (%)	2 (0.3)	1 (0.2)	0 (0)	0.43
LPF† – n (%)	4 (0.6)	6 (1.0)	3 (0.4)	0.5
ITT–ACPR – n (%)	628 (94.4)	593 (95.8)	664 (97.9)	0.004
PP–ACPR – n/*N* (%)	628/634 (99.1)	593/601 (98.7)	664/667 (99.6)	0.25
Reinfection rate – n/*N* (%)	231/634 (36.4)	117/601 (19.5)	62/667 (9.3)	< 0.001

*Possible failure: lost to follow-up or withdrawal or subjects for which filters were missing or undetermined PCR.
†These are computed according to ITT analysis.
PCR = polymerase chain reaction; ITT = intention-to-treat; PP = per protocol; AL = artemether-lumefantrine; AS+AQ = artesunate plus amodiaquine; AS+SP = artesunate plus sulfadoxine-pyrimethamine; n/*N* = frequency; ETF = early treatment failure; LCF = late clinical failure; LPF = late parasitological failure; ACPR = adequate clinical and parasitological failure.

TABLE 4

Incidence of adverse events* occurred within 3 days after treatment initiation

Symptoms/signs	AL n/*N* (%)	AS+AQ n/*N* (%)	AS+SP n/*N* (%)	*P* value
Vomiting	25/281 (8.9)	58/269 (21.6)	67/289 (23.2)	< 0.001
Headaches	2/30 (6.7)	7/39 (18.0)	5/49 (10.2)	0.36
Asthenia	0/113	1†/123 (0.81)	0/135	0.64
Dizziness	0/129	2/132 (1.5)	1/146 (0.7)	0.53
Anorexia	0/109	3/123 (2.4)	2/140 (1.4)	0.33
Abdominal pain	8/100 (8.0)	8/85 (9.4)	9/104 (8.7)	0.94
Rash	1/654 (0.15)	0/612	0/666	0.65

*These adverse events are considered to be related to the study drugs because of the temporal relationship with the study drug although the possibility that these symptoms can also be caused by malaria.

†The subject was having difficulty walking during the asthenia episode.

AL = artemether-lumefantrine; AS+AQ = artesunate plus amodiaquine; AS+SP = artesunate plus sulfadoxine-pyrimethamine; n/N = frequency.

(in AS+SP arm); 2 cases of death in AS+SP arm (one caused by pneumonia and the other to hypoglycemia). The occurrence of SAE was comparable in the three arms. All of the above non-fatal SAE resolved without sequelae. The two cases of death happened in the AS+SP arm, although a causal relationship with the study product was not established. All SAEs were assessed as not related to the study drugs. There was no significant laboratory abnormalities (hemogramme, creatinemia, and alanine aminotransferase parameters) related to any of the study treatment drugs (Table 4).

DISCUSSION

Over 24 months of follow-up of this study show that the incidence of uncomplicated malaria was significantly lower in the AS+SP and AS+AQ arms than in the AL arm. This could be explained by the lowest re-infection rate seen in the AS+SP and AS+AQ arms and maybe because of the longer half life of the partner drugs[7] (AQ or SP) or else to increasing resistance to lumefantrine. However, *in vitro* studies conducted during the same period in Mali did not show any lumefantrine resistance.[22]

Our results are in contrast with the result of a study conducted in Ghanaian children[15] with repeated treatment of AS+AQ or AL where a similar incidence rate between the two study arms was reported. This may be because in our study, we counted late parasitological failures caused by re-infections (as determined by PCR) as new malaria episodes. As in our study, the author reported good safety profile and high 28-day follow-up treatment efficacy in both arms (AS+AQ and AL). We found that malaria incidence was high in children < 5 years of age but also in children > 5 years of age compared with adults.

The high PCR-corrected 28-day follow-up treatment efficacy results are consistent with reports from the same area in 2004 where ACPRs rates of 100% and 99.1% were found with AS+SP and AS+AQ, respectively.[7] Our data are similar to those of Senegal[17] and Indonesia with AS+SP[23] and a multicenter study in Africa with AL and AS+AQ.[24]

TABLE 5

Incidence of laboratory adverse events* occurred within 28 days after treatment initiation

Laboratory adverse events	AL n/*N* (%)	AS+AQ n/*N* (%)	AS+SP n/*N* (%)	*P* value
ALAT	4/568 (0.72)	4/537 (0.74)	7/592 (1.18)	0.68
Creatinemia	27/530 (5.09)	31/490 (6.33)	84/553 (15.19)	< 0.001
Leucopenia	2/578 (0.35)	1/551 (0.18)	5/600 (0.83)	0.30

*Laboratory values normal on Day 0 but abnormal during follow-up. All incident cases of leucopenia were grade 1 (mild; < 3,500–2,500 leukocytes mm^3).

AL = artemether-lumefantrine; AS+AQ = artesunate plus amodiaquine; AS+SP = artesunate plus sulfadoxine-pyrimethamine; ALAT = alanine aminotransferase; n/*N* = frequency.

The re-infection rate was higher in the AL arm as compared with AS+AQ and AS+SP arms ($P < 0.001$). This high reinfection rate with AL is in contrast with studies in Uganda[25] and Tanzania[13] where lower reinfection rates were reported in AL compared with AS+AQ. The authors thought that the higher reinfection rates in AS+AQ could be explained by the relatively high amodiaquine resistance level in the study area. This study found that the risk of recurrent parasitemia (uncorrected PCR 28-day follow-up ACPR) malaria had a significant seasonal variation. We found significant recurrent parasitemia during the rainy season compared with the dry season. This study finding is consistent with a Ugandan study,[25] which also found significant seasonal variation in recurrent parasitemia despite the perennial malaria transmission pattern in that study setting. The significant recurrent parasitemia during the rainy season compared with the dry season could be explained by the high intensity of the malaria transmission during the rainy season compared with the dry season.

Our participants received quinine as a second-line treatment. However, it is unclear whether quinine, the same ACT, or a different ACT would be the optimal treatment. Given that nearly all recurrent parasitemia were caused by new infections, it might have been reasonable to retreat with the same ACT regimen, rather than with quinine.

The repeated treatment using the same ACT in this study showed a good safety profile. This finding is consistent with other studies that also reported a good safety profile of repeated treatment of ACT,[13–15,17] even in chronically malnourished children with or without HIV infection.[14] Although AL was associated with a higher reinfection rate and hence higher malaria incidence rate, it did produce fewer side effects such as vomiting.

Limitations of this study include the consideration of asymptomatic parasite carriers from Day 7 during each 28-day treatment follow-up as treatment failures (as in the WHO treatment assessment guidelines[19]), which were treated with quinine and counted as malaria episodes for new infections as determined by PCR. The overall malaria episodes may be similar if one considered only symptomatic parasite recurrence during each 28-day treatment follow-up and the clinical episodes occurring afterward. Although our sample size calculation assumptions expected differences equal or greater than 35%, we observed statistically significant differences in malaria incidence reduction of 16% and 20% with AS+AQ and AS+SP, respectively, compared with AL. This is probably because our original calculation was conservative and yielded a rather large sample size, resulting in smaller differences being statistically significant. In addition, the mean malaria incidence was higher than expected (two episodes expected but almost three episodes observed)

We show that ACTs that had very high and similar efficacy and safety by the standard 28-day drug efficacy test could have a different impact on malaria incidence over a longer period. In this era of high efficacy of ACTs, the choice of a first-line treatment policy should go beyond short-term efficacy and safety to include longer follow-ups and repetitive treatments with the same regimen. Additional studies will be

required to assess the overall impact of the large-scale deployment of ACTs on malaria in the field.

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III. Méthodes statistiques appropriées applicables pour l'analyse des données récurrentes de paludisme

Au cours du suivi des patients, plusieurs accès palustres surviennent à des intervalles de temps différents. Le délai entre 2 accès dépend, entre autres, de la cinétique du traitement, de l'environnement (lieu de vie), des mesures prophylactiques misent en œuvres. Tenir compte des récurrences et des délais permet de mieux apprécier l'efficacité thérapeutique.

Lors de la conduite de l'essai thérapeutique, le patient recevait le même traitement antipaludique initial reçu à la randomisation à chaque épisode de paludisme durant toute la durée de l'étude.

Quatre modèles ont été utilisés pour l'analyse de ces événements récurrents: i) Les équations d'estimation généralisées (GEE) utilisant la distribution de Poisson; et trois autres modèles qui sont une extension du modèle Cox: ii) le modèle de processus de comptage d'Andersen-Gill (AG-CP), iii) le modèle de processus de comptage de Prentice-Williams-Peterson (PWP-CP); et iv) le modèle de fragilité. Pour tenir compte de la nature récurrente des données, les modèles i) à iii) sont des modèles marginaux et le modèle iv) utilise un terme de fragilité partagée, modélisant des intervalles de temps continus ou discontinus entre les accès. Pour l'analyse de risque à intervalles de temps discontinus, 14 jours de fenêtre étaient considérés comme temps où le patient est supposé ne pas être à risque d'un nouvel épisode après le début d'un traitement de paludisme, ceci du fait de la pharmacocinétique des médicaments utilisés. Les modèles étaient comparés en utilisant la magnitude des risques relatifs (RR) estimés et de leurs écarts types.

Les résultats de l'analyse indiqués dans les Tableau 2 et 3 de nos travaux publiés en 2014 dans « *Method* » de « *Malaria Journal*» dont l'article est joint ci-dessous, indiquaient que les modèles d'AG-CP et de fragilité partagée ont montré d'effets significatifs et similaires de

traitements (comparativement au traitement de référence) sur les épisodes récurrents de paludisme, ceci aussi bien avec le modèle de risque à intervalles discontinus que continus.

Article 2: « Modelling recurrent events: comparison of statistical models with continuous and discontinuous risk intervals on recurrent malaria episodes data »

MALARIA JOURNAL

METHODOLOGY Open Access

Modelling recurrent events: comparison of statistical models with continuous and discontinuous risk intervals on recurrent malaria episodes data

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Abstract

Background: Recurrent events data analysis is common in biomedicine. Literature review indicates that most statistical models used for such data are often based on time to the first event or consider events within a subject as independent. Even when taking into account the non-independence of recurrent events within subjects, data analyses are mostly done with continuous risk interval models, which may not be appropriate for treatments with sustained effects (e.g., drug treatments of malaria patients). Furthermore, results can be biased in cases of a confounding factor implying different risk exposure, e.g. in malaria transmission: if subjects are located at zones showing different environmental factors implying different risk exposures.

Methods: This work aimed to compare four different approaches by analysing recurrent malaria episodes from a clinical trial assessing the effectiveness of three malaria treatments [artesunate + amodiaquine (AS + AQ), artesunate + sulphadoxine-pyrimethamine (AS + SP) or artemether-lumefantrine (AL)], with continuous and discontinuous risk intervals: Andersen-Gill counting process (AG-CP), Prentice-Williams-Peterson counting process (PWP-CP), a shared gamma frailty model, and Generalized Estimating Equations model (GEE) using Poisson distribution. Simulations were also made to analyse the impact of the addition of a confounding factor on malaria recurrent episodes.

Results: Using the discontinuous interval analysis, AG-CP and Shared gamma frailty models provided similar estimations of treatment effect on malaria recurrent episodes when adjusted on age category. The patients had significant decreased risk of recurrent malaria episodes when treated with AS + AQ or AS + SP arms compared to AL arm; Relative Risks were: 0.75 (95% CI (Confidence Interval): 0.62-0.89), 0.74 (95% CI: 0.62-0.88) respectively for AG-CP model and 0.76 (95% CI: 0.64-0.89), 0.74 (95% CI: 0.62-0.87) for the Shared gamma frailty model.
With both discontinuous and continuous risk intervals analysis, GEE Poisson distribution models failed to detect the effect of AS + AQ arm compared to AL arm when adjusted for age category. The discontinuous risk interval analysis was found to be the more appropriate approach.

Conclusion: Repeated event in infectious diseases such as malaria can be analysed with appropriate existing models that account for the correlation between multiple events within subjects with common statistical software packages, after properly setting up the data structures.

Keywords: Recurrent events, Malaria, Discontinuous risk intervals, Extended Cox model, Shared frailty model, GEE

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Background

Recurrent events data analysis is quite common in biomedicine, such as low back pain, sick leave from work, sporting injuries, hospital readmissions and episodes of infectious diseases such as malaria [1-7]. Literature review indicates that most statistical models applied to such data are often based on naive techniques. Such naive techniques are characterized by either ignoring the existence of recurrent events, or ignoring the fact that the recurrent events within subjects are correlated [1,2]. Even when taking into account the non-independence of recurrent events within subjects, data analyses are mostly done with continuous risk interval models [2-5], which may not be relevant for health conditions with discontinuous risk [8]. In the medical field, it is quite common to encounter recurrent health conditions with such discontinuous risk intervals, e.g. in cases with persistent treatment effect. Examples include infections, such as malaria, disability episodes, hospitalizations, and nursing home admissions [7-12]. When subjects have a disability episode, they are not at risk of the second episode of disability until they have recovered from the first episode. To obtain unbiased estimates of incidence rates, the person-time period when the subject is not at risk should be excluded from the risk set. When analysing recurrent time-to-event outcomes with discontinuous risk intervals the subject is not at risk of another event while a previous one is ongoing or if the subject is under treatment. Appropriate models for analysing recurrent events data include marginal models or frailty or random coefficient analysis models [8-13], which take into account the non independence assumption of events within the subject.

Furthermore, in the case of malaria treatment trials, investigators assume that randomization is sufficient for controlling differential risk. However, the location of each subject is important as the risk exposure shows high spatial variations due to different environmental factors [14,15] that must be taken into account.

This work aimed at comparing different approaches analysing recurrent malaria episodes, with continuous and discontinuous risk interval models, in order to contribute identifying useful models to analyse such malaria data.

Methods

Study design

Data were collected from July 2005 to July 2007 in Bougoula-Hameau, Sikasso, in the south region of Mali. Patients were randomized as they came to the health centre from July 2005 to October 2006 (accrual period) to one of the artemisinin-based combination therapy (ACT) arm: artesunate + amodiaquine (AS + AQ) or artesunate + sulphadoxine-pyrimethamine (AS + SP) or artemether-lumefantrine (AL). The study's main objective was to compare malaria incidence between these three ACT. Patients received the same initial treatment at each subsequent episode of uncomplicated malaria during the course of the study. The clinical data and results related to the main objective were published elsewhere [7].

Statistical models and data analysis

Four models were used for the analysis of recurrent time-to-event outcomes: i) Generalized estimating equations (GEE) model using a Poisson distribution; and three extended Cox models: ii) the Andersen-Gill counting process (AG-CP), iii) the Prentice-Williams-Peterson counting process (PWP-CP); and iv) the Shared gamma frailty model. To take into account the recurrent structure of the data, models i) to iii) are marginal and model iv) uses a shared frailty term.

Both continuous and discontinuous time interval approaches were used with the four different models to analyse the data. A 14 day washout period was estimated after each episode treatment based on the pharmacokinetics of the study drugs. Model results were evaluated by comparing the risk ratio (RR) estimates and their standard errors (SE).

To analyse the impact of a confounding factor on results, a simulation of a risk exposure was done with two levels (high risk exposure *versus* low risk exposure) using a Binomial distribution with parameter (probability of being in the high risk exposure class) depending on the number of malaria episodes: 0.99 if the subject experienced >6 malaria episodes else 0.75 if the subject experienced > 4 malaria episodes, else 0.25 if subject experienced >1 episode and otherwise 0.01. This binary factor simulates, for example, two zones of different exposure. For analysing the impact of such a confounding factor, 1,000 independent replicates were performed, using R2.15.2 software (The R Foundation for Statistical Computing, Vienna, Austria). With the simulation data, only discontinuous time interval analysis was done with each of the four models. The impact of the simulated confounding factor on the results for each model was assessed by comparing the magnitude and confidence intervals of RR estimates, power and empirical coverage rates (ECR). For each covariate, the ratio between the standard error (SE) of the estimates using simulated data and the SE of the estimates using the observed data was computed. These criteria can be interpreted as the impact of the confounding factor on the estimation accuracy.

GEE model using Poisson distribution

The Poisson regression model is frequently used to analyse count data or to study disease incidence and mortality when the dependent variable represents the number of independent events that occur during a fixed period

of time [16]. The conditional mean of Y (number of events) can be written as:

$$Ln(Y|X,\beta) = X_i\beta \quad (1)$$

Where, $X_i\beta = \beta_0 + \beta_1X_1 + \beta_2X_2 + \ldots + \beta_nX_n$; Ln is the natural logarithm (the canonical link between the linear predictors and the conditional mean of Y).

The GEE Poisson estimates the same model as the standard Poisson regression allowing for dependence within clusters. Therefore, it is appropriate to model recurrent events within a subject, such as in longitudinal data. The regression coefficients are refit, correcting iteratively for the correlation. In such models, the within-subject correlation structure is treated as a nuisance parameter. In this work the exchangeable correlation structure has been used assuming that the correlation between events remained constant through the time [17].

Extended Cox models

The extended Cox models are used to model recurrent events within a subject unlike the Cox model, which is used to model a unique event or, sometimes, the first event. The considered Cox extended models were: the counting process model (Anderson-Gill model or AG-CP) [18], and the conditional model (Prentice-Williams-Peterson counting process model or PWP-CP) [16].

The sandwich robust standard error of Lin and Wei [3,19], which is a variance-correction technique, is usually employed together with these Cox extended models to avoid inflation of type I error due to multiple observations per individual which do not require specification of the correlation matrix.

The Anderson-Gill model (AG-CP)

The formula is written as:

$$\lambda_{ik}(t/X,\beta) = \mathbb{I}_{ik}(t)\lambda_0(t)e^{X_{ik}\beta} \quad (2)$$

$\lambda_{ik}(t)$ represents the hazard function for the k^{th} event of the i^{th} subject at time t; $\lambda_0(t)$ represents the common baseline hazard for all events over time; X_{ik} represents the vector of p covariates processes for the i^{th} individual; β is a fixed vector of p coefficients; $\mathbb{I}_{ik}$ is a predictable process, taking values in {1,0} indicating when the i^{th} individual is under observation.

The AG-CP model uses this counting process time-scale for all episodes. The time-scale does not reset to 0 after an episode (Table 1). Data for each subject needs to be entered in the counting process style, with a start time, stop time and censoring indicator for each event.

Conditional model (Prentice-Williams-Peterson counting process-PWP-CP):

The PWP-CP model is similar to the AG-CP model but stratified by events. The formula is written as:

$$\lambda_{ik}(t|X,\beta) = \mathbb{I}_{ik}(t)\lambda_{0k}(t)e^{X_{ik}\beta} \quad (3)$$

$\lambda_{0k}(t)$ represents the event-specific baseline hazard for the k^{th} event over time. In this model, a subject is assumed not to be at risk for a subsequent event until a current event has terminated.

The shared frailty model

The frailty model, introduced in the biostatistical literature by Vaupel *et al.* [20], and discussed in detail by Hougaard, Duchateau and Janssen, and Wienke *et al.* [21-23], accounts for the heterogeneity in baseline. This model is an extension of the proportional hazards model in which the hazard function depends upon an unobservable random variable. Subjects may be exposed to different risk levels, even after controlling for known risk factors, because of some relevant unobserved covariates. The frailty parameter models these unknown covariates. In a shared frailty model, individuals in the same group share the same frailty value which generates dependence between those individuals who share frailties.

The shared frailty model can be written as follows:

$$\lambda_{ik}(t/X,\beta,u) = u_i\lambda_{ik}(t) = \lambda_0(t)e^{X_{ik}\beta+u_i} \quad (4)$$

Where λ_{ik} is the conditional hazard function for the k^{th} subject from the i^{th} cluster (conditional on u_i); $\lambda_0(t)$ is the baseline hazard; β is the fixed effects vector of dimension p; X_{ik} is the vector of covariates; u_i is the random effect for the i^{th} cluster. Subjects in the same cluster u share the same frailty factor [22]. It is a conditional hazard model, given the u_i. The cluster may represent a family, for example, or as in this case a single subject for which multiple episodes are observed.

The distribution of u may be Gamma, Gaussian, or other distribution. The gamma distribution has been chosen because of its mathematical tractability and because it is widely used [22]. The one-parameter chosen gamma distribution is defined as:

$$f_w(u) = \frac{\nu^{1/\theta-1}e(-u/\theta)}{\theta^{\frac{1}{\theta}}\Gamma(1/\theta)} \quad (5)$$

with Γ the gamma function. Note that $E(u) = 1$ and $Var(u) = \theta$. This gives the following interpretation: subject in a class i with $u_i > 1$ are frail, meaning of higher risk while subject with $u_i < 1$ are strong, meaning of lower risk. The parameter θ informs on the clusters or classes heterogeneity in the population.

Table 1 Data structures for modelling recurrent time-to-event outcomes

ID	Start	End	Episode	Order	Time	Treatment	Age (Years)	Quarter
1	0	28	1	1	28	AS + SP	3.93	1
1	42	52	1	2	10	AS-SP	3.93	1
1	476	700	0	10	224	AS + SP	3.93	1
2	0	77	1	1	77	AS + AQ	1.15	1
2	91	375	1	2	284	AS + AQ	1.15	1
2	417	700	0	4	283	AS + AQ	1.15	1
3	0	28	1	1	28	AL	1.48	1
3	42	78	1	2	36	AL	1.48	1
3	150	700	0	5	550	AL	1.48	1

Data dictionary:
ID: study subject identification number; start: the start time of the interval (in days); end: the time (in days) at which the event occurs or the time of censoring; episode, the occurrence of malaria episode (yes = 1, no = 0); order: the order of the episodes, which is used only for the PWP-CP model; time: the number of days at risk that is calculated from subtracting end from start variables; treatment: the same malaria treatment given to the patient during each episode; Age (Years): the patients age at enrolment in years; quarter: the resident place or bloc of the patient in the village (old quarter = 1, new quarter = 0).

As in the Cox model or its extensions, the baseline hazard function for the frailty model does not vary by event, but the coefficient estimates of covariates effect from the frailty model, unlike the Cox model may vary if there is a significant random effect.

For the baseline hazard function, although other distribution could be used, the Weibull proportional hazards distribution was assumed. Weibull distributed event times are often used in practice, because they are able to describe the actual evolution of the hazard function in an appropriate way in many circumstances. Furthermore it is a popular flexible parametric model that allows the inclusion of covariates of the survival times [22].

Data structure

The duration of each subject in the study was defined as the time between enrolment and the end of the study or until the subject is lost to follow or withdrawn. A malaria episode (event) had to be preceded and followed by a time period without malaria except in the case of withdrawal and at the end of the study period. As an example, Table 1 provides data for three study subjects (one in each study arm). Subject 1 had nine malaria episodes at days 28, 52, 80, 109, 305, 326, 410, 438 and 462 and the follow-up ended on day 700. Subject 2 had three malaria episodes at days 77, 375 and 403 and the follow-up ended on day 700. Subject 3 had four malaria episodes at days 28, 78, 105 and 136 and the follow-up ended on day 700. The duration of each malaria episode is 14 days as it is assumed that the subject is not at risk of new infection for this duration after treatment initiation (discontinuous risk interval data structure).

The data are organized as one record per subject per event. The data structure for the AG-CP model consists of the first four columns. A subject with multiple events is considered as multiple subjects for analytic purposes. For example, subject 2 is considered four times: the first begins follow-up at time 0 and has an event at 77 days; according to the fact that the subject is not at risk during 14 days, the second has delayed entry at 91 days and has an event at 375 days; the third has delayed entry at 389 days and has an event at 403 and is followed through 700 days without having an event. Because the counting process model does not consider the order of the events, it does not use the "order" column. In the PWP-CP model, a subject is assumed not to be at risk for a subsequent event until the current event has terminated. This means, one cannot be at risk for the second event without having experienced and completed the first event. The data structure for PWP-CP model is similar to that of the counting process AG-CP model except that the "order" column is also used to identify the event order. An Additional file 1 shows statistical codes for each model using Stata and R Software [see Additional file 1].

Incidence rate and relative risk estimate of recurrent events

The incidence rate was computed as the number of events per person-days [24] and can be calculated from Table 1 as follows:

$$\frac{\sum_{k=1}^{n} event_{ij}}{\sum_{k=1}^{n} time_{ij}}$$

where $event_{ij}$ is the event status (1 or 0) for the i^{th} subject in the j^{th} interval; $time_{ij}$ is the time at risk for the i^{th} subject in the j^{th} interval; n is the number of subjects. The relative risks (RRs) were computed to assess the treatment effect (AL study arm was used as the reference treatment)

and the age group effect (age group < 5 years old, 5-9 years old and >9 years old. The age group >9 years old was used as reference group) on the occurrence of malaria episode. The hazard ratio (HR) for extended Cox models and the relative risk (RR) for the GEE model were estimated.

Ethical considerations

The study has been approved from the institutional ethical committee (FWA #00001769) at the Faculty of Medicine, Pharmacy and Odonto-Stomatogy (FMPOS)/USTTB, Bamako, Mali.

A written consent was also obtained from each participant or their parent/legal guardian.

Results

From July 2005 to July 2007, the 777 subjects enrolled into the study yielded a total of 1,649 malaria episodes (min = 1, max = 12, median = 2 episodes per subject). Using the discontinuous risk interval analysis, PWP-CP, AG-CP, and the Shared gamma frailty models provided larger treatment effect on malaria episodes compared to GEE for the patients treated with AS + AQ or AS + SP as compared to the AL arm; RRs were: 0.75 (95% CI (Confidence Interval): 0.62-0.89), 0.74 (95% CI: 0.62-0.88) respectively for AG-CP model, 0.76 (95% CI: 0.64-0.89), 0.74 (95% CI: 0.62-0.87) for the Shared gamma frailty model and 1.02 (0.93-1.11), 0.93 (0.87-0.99) for GEE model (Table 2). Similarly for the age category, using the discontinuous (Table 2) risk interval analysis, PWP-CP, AG-CP and the Shared gamma frailty models provided similar and higher magnitude of RRs for the patients in age group <5 years old or age group between 5-9 years old compared to patients of age group >9 years old; RRs were: 3.16 (95% CI: 2.15-4.65), 2.61 (95% CI: 1.76-3.88) respectively for AG-CP model and 3.04 (95% CI: 2.27-4.09), 2.54 (95% CI: 1.87-3.45) respectively for Shared gamma frailty model. The effect of covariates (treatments and age category) on malaria episodes were slightly higher for both AG-CP and the Shared gamma frailty models in discontinuous risk intervals (Table 2) compared to continuous risk intervals (Table 3).

Using both discontinuous (Table 2) and continuous (Table 3) risk intervals analysis, GEE Poisson models did not find a preventive efficacy for the patients treated with AS + AQ arm compared to AL arm. Furthermore, the GEE models estimated a protective efficacy (1-RR) of lower magnitude for AS + SP than the 3 other models. The discontinuous (Table 2) and continuous (Table 3) risk intervals analysis results were similar for GEE Poisson model.

Incidence rates (Table 4) were slightly higher in the discontinuous interval analysis compared to the continuous interval analysis as the person-time was lower, although the 5% significance level was not reached for the incidence rate differences between treatment groups (exact mid p-values).

Assessing the impact of risk exposure covariate with the simulated data (Figure 1), treatment effect estimates (AS + AQ and AS + SP compared to AL) were relatively lower than those with observed data for the AG-CP and the Shared gamma frailty models, but still remain significant for each extended Cox models. For the GEE model, there were no significant treatment at 5% for both AS + AQ and AS + SP compared to AL. Simulated data (Figure 1) confirmed the significant (at 5% significant level) treatment effect of AS + AQ and AS + SP compared to AL with power > 80% for all models except the GEE Poisson distribution model. Also, the age category effects (age category < 5 years and age category 5-9 years old compared to the age category > 9 years old) on malaria episodes were relatively lower compared to the effects of the age category on malaria episodes using the observed data analysis. For the GEE model, there were moderate to no significant age category effects.

The simulated data (Figure 1) showed that adding a significant covariate reduces estimation variances. For the AS + AQ treatment, the standard error ratio (simulated over observed) for the AG-CP model was 0.72 (2.5-97.5 percentile [0.60-0.85]), while it was 0.16 (2.5-

Table 2 Coefficient estimates according to model by discontinuous risk intervals analysis

Models	AS + AQ* RR/HR (SE); [95% CI]; *p*	AS + SP* RR/HR (SE); [95% CI]; *p*	<5 years** RR/HR (SE); [95% CI]; *p*	5-9 years** RR/HR (SE); [95% CI]; *p*
GEE, Poisson distribution	1.02 (0.044); [0.93-1.11]; p = 0.722	0.93 (0.029); [0.87- 0.99]; p = 0.018	1.36 (0.201); [1.02-1.82]; p = 0.036	1.22 (0.181); [0.91-1.63]; p = 0.175
AG-CP	0.75 (0.068); [0.62-0.89]; p < 0.001	0.74 (0.065); [0.62-0.88]; p < 0.001	3.16 (0.621); [2.15-4.65]; p < 0.001	2.61 (0.526); [1.76-3.88]; p < 0.001
PWP-CP	0.86 (0.055); [0.76-0.97]; p = 0.015	0.85 (0.052); [0.75-0.96]; p = 0.007	2.34 (0.389); [1.69-3.24]; p < 0.001	2.04 (0.345); [1.46-2.84]; p < 0.001
Shared gamma frailty	0.76 (0.064); [0.64-0.89]; p = 0.001	0.74 (0.063); [0.62-0.87]; p < 0.001	3.04 (0.458); [2.27-4.09]; p < 0.001	2.54 (0.397); [1.87-3.45]; p < 0.001

*Reference treatment: AL.
**Reference age group: >9 years old.
CI: Confidence interval for RR/HR (Relative risk/Hazard ratio); SE: Standard error; p: p value.

Table 3 Coefficient estimates according to model by continuous risk intervals analysis

Models	AS + AQ* RR/HR (SE); [95% CI]; *p*	AS + SP* RR/HR (SE); [95% CI]; *p*	<5 years** RR/HR (SE); [95% CI]; *p*	5-9 years** RR/HR (SE); [95% CI]; *p*
GEE, Poisson distribution	1.02(0.044); [0.93-1.11]; p = 0.722	0.93 (0.029); [0.87- 0.99]; p = 0.02	1.36 (0.201); [1.02-1.82]; p = 0.04	1.22 (0.181); [0.91-1.63]; p = 0.18
AG-CP	0.77 (0.064); [0.65-0.91]; p = 0.002	0.76 (0.062); [0.65-0.89]; p = 0.001	2.94 (0.559); [2.03-4.27]; p < 0.001	2.48 (0.481); [1.69-3.62]; p < 0.001
PWP-CP	0.83 (0.053); [0.73-0.94]; p = 0.004	0.81 (0.050); [0.72-0.92]; p = 0.001	2.35 (0.388); [1.71-3.26]; p < 0.001	2.05 (0.344); [1.48-2.85]; p < 0.001
Shared gamma frailty	0.77 (0.061); [0.66-0.90]; p = 0.001	0.76 (0.060); [0.65-0.89]; p < 0.001	2.88 (0.415); [2.17-3.82]; p < 0.001	2.43 (0.364); [1.82-3.26]; p < 0.001

*Reference treatment: AL.
**Reference age group: >9 years old.
CI: Confidence interval for RR/HR (Relative risk/Hazard ratio); SE: Standard error; p: p value.

97.5 percentile [0.13-0.24]) for GEE Poisson distribution model. For the AS + SP treatment, the standard error ratio (simulated over observed) for the AG-CP model was 0.75 (2.5-97.5 percentile [0.63-0.89]), while it was 0.25 (2.5-97.5 percentile [0.23-0.54]) for GEE Poisson distribution model. The AS + SP effect estimates was clearly modified when using the GEE model (ECR = 7.7%), similarly to small age categories effect estimates when using the Shared gamma frailty model (ECR = 16.1%). AG-CP and PWP-CP models estimates were particularly stable after the addition of the simulated confounding covariate.

Discussion

Methods are available to analyse data with recurrent events while accounting for the lack of independence among events [3-6,8-13]. In this paper, when focusing on recurrent malaria episodes data, results were different according to the model used, highlighting the importance of the model choice according to the medical question studied and the collected dataset. Two other survival models for recurrent events [3,8,20] have been proposed: Wei-Lin-Weissfeld (WLW), Prentice-Williams-Peterson gap time (PWP-GT). Various earlier studies have discussed the difficulty in conceptualizing the risk set of the WLW model and the biases in the estimates from the WLW and PWP-GT models [3,6,25], so these two models were not considered here. There is a limited applied statistical research modelling malaria recurrent episodes data [12], though malaria is one of the most devastating diseases in sub-Saharan Africa. The literature review indicates that, this is the first applied statistical research modelling using the main extended Cox models on recurrent malaria episodes data with both discontinuous and continuous risk intervals. The data structures have been efficiently prior organized with discontinuous risk intervals in order to perform the analysis using these different models. For these analyses, the time while the subject is not at risk because of the malaria treatment has to be taken into account and excluded from the risk set. There is a need of careful preparation of the data structure before applying theses analyses.

The AG-CP and the Shared gamma frailty models, with both discontinuous and continuous risk intervals provided similar parameter estimates of treatment effects (with respect to the referent treatment) or for age category effects (in respect to age referent category) on malaria recurrent episodes. This was previously observed by others [8] where the authors reported significant covariate effect estimates using AG-CP and the Shared gamma frailty models with discontinuous risk intervals analysis. AG-CP models are known to be useful and robust if one is interested in the overall effect, such as the treatment effect in a clinical trial, and if there is no clear biological mechanism underlying the relation between the first event and subsequent events [8,11]. The actual used data seems adapted to this described situation where having the first malaria episode does not preclude subsequent malaria episode as long as exposure is present

Table 4 Incidence rate* per treatment arm according to discontinuous or continuous risk intervals analysis

Model	AL	AS + AQ	AS + SP
	IR [95% CI]	IR [95% CI]	IR [95% CI]
Discontinuous time risk intervals	2.01 [1.86-2.17]	1.52 [1.39-1.66]	1.50 [1.37-1.64]
Continuous time risk intervals	1.87 [1.73-2.02]	1.44 [1.32-1.57]	1.42 [1.30-1.55]
p-value	0.09	0.19	0.19

*Malaria episodes/person/year.
IR: Incidence rate; CI: Confidence interval for IR; *p-values*: Exact mid p-values for risk difference between discontinuous and continuous risk intervals.

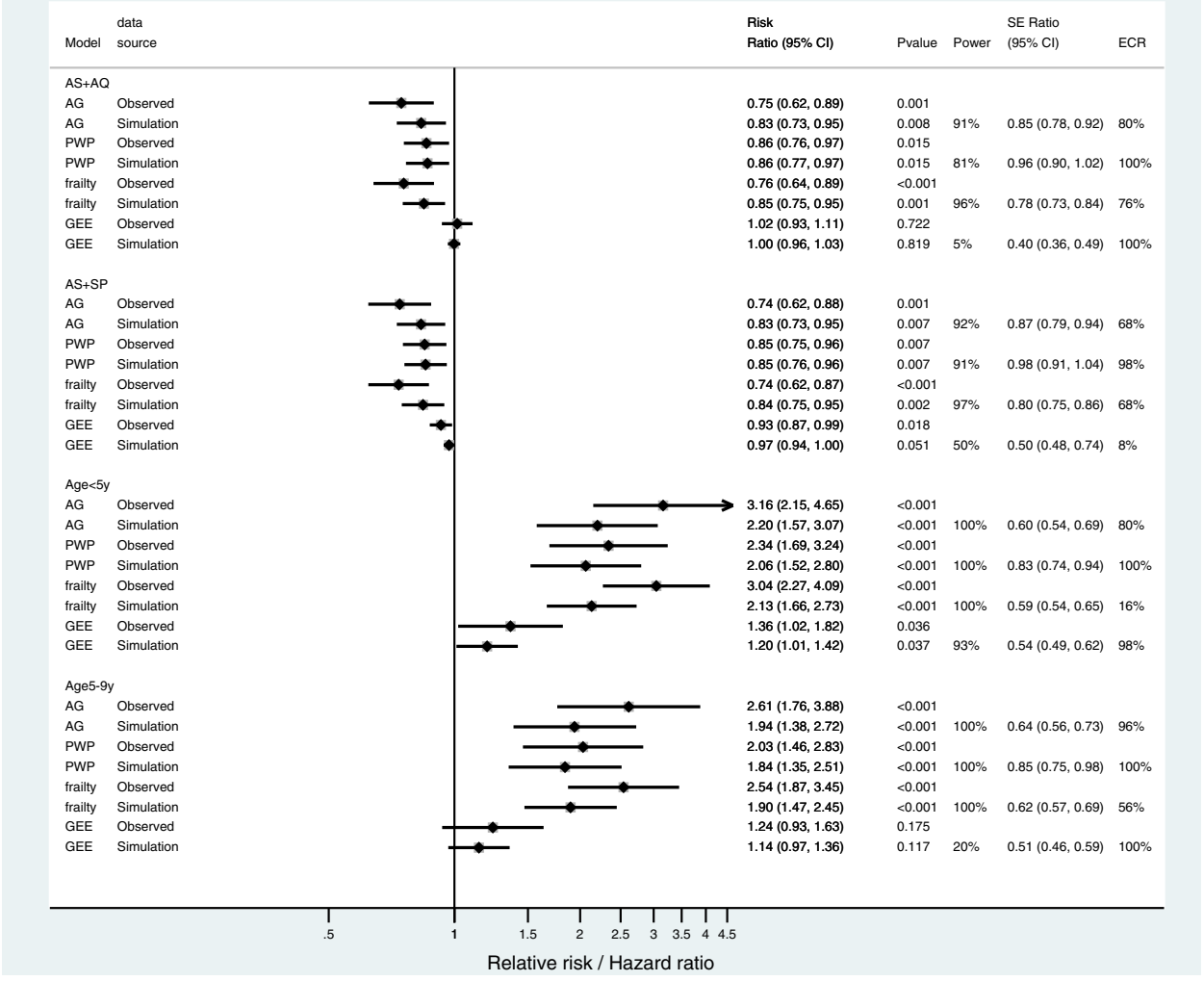

Figure 1 Coefficients estimate using observed and simulated data using discontinuous risk intervals analysis. Dark dots with lines are Relative risk/Hazard ratio and their 95% Confidence intervals respectively; Vertical central dark line is either the no effect treatment line compared to the referent category (artemether-lumefantrine) or the no effect age group compared the referent category (age group > 9 years old). Abbreviations: AS + AQ, artesunate + amodiaquine; AS + SP, artesunate + sulphadoxine-pyrimethamine; AG, Andersen-Gill; PWP, Prentice-Williams-Peterson; GEE, generalized estimating equation; SE, standard error; ECR, empirical coverage rate.

(transmission condition), and is taken into account. The counting process (or AG-CP) model requires few assumptions, and is comparably robust like the traditional Cox regression model [3,18]. Although the Shared gamma frailty models generated similar results to the AG-CP model in this study, the specification of the frailty distribution may affect the coefficient estimates and more research is still needed in this area [8,22].

AS + SP, then followed by AS + AQ showed significant protective effects (1-RR) against recurrent malaria episodes compared to AL using the three extended Cox models (AG-CP, PWP-CP and the Shared gamma frailty). This could be explained by the longer half-life of the partner drugs (AQ and SP) and this observation has been reported previously [7]. In contrast, both the age category of <5 years old and the age category of 5-9 years old were significantly at higher risk of recurrent malaria episodes compared to old children and adults (>9 years old) using the three extended Cox models (AG-CP, PWP-CP and the Shared gamma frailty).

Time discontinuous or continuous risk intervals models should be chosen based on the disease or outcome conditions. In this case, the discontinuous risk intervals model should be chosen for unbiased estimates of covariate coefficients as shown in these data and also reported previously [8]. It is also more appropriate from an epidemiological point of view to take into account the time a subject is not at risk for a disease in a given period of time.

Less or no significant covariate effects (with respect to referent category) were found on malaria recurrent episodes using GEE for both discontinuous and continuous risk intervals models which yielded similar coefficient estimates. GEE is considered a very flexible approach [26]. Indeed, GEE models can handle a variety of correlated measure models that arise from recurrent episodes measure in the same individuals over time [27-29]. GEE models are robust to misspecification of the correlations structures [26]. Selecting robust standard errors (Huber/White Sandwich Estimators; as opposed to conventional standard errors) allow the estimates to be valid even if the correlation structure is misspecified. The poor performance of GEE, shown here, may also be explained by inappropriate assumption of the Poisson distribution [30] as the data maybe underdispersed with 70% of the dependant value count greater than zero.

Analyses including the simulated variables also showed significant treatments (AS + AQ and AS + SP using AL as referent category) and age category (using >9 years old as referent category) effects on malaria recurrent episodes with AG-CP, PWP-CP and the Shared gamma frailty models as in observed data analysis though these effects were more important for AG-CP and the Shared gamma frailty models.

The simulation study showed that a risk exposure factor modified the estimates, reducing the treatment effects. In malaria studies conducted in sub-Saharan countries, the main risk exposure factor includes the house location of subjects [14,15] due to specific environments, which influence malaria transmission. The distribution of mosquito breeding sites within villages is not uniform, thus, the exposure varies within villages, and this must be taken into account when assessing treatment effects. As shown in Figure 1, the simulation data provided proof of an existing confounding factor that needs to be taken into account. In fact, the RRs and their confidence intervals estimated on simulated data trended toward the null hypothesis (more close for RRs confidence intervals to include 1) compared to the RRs and their confidence intervals estimated on observed data. These variations were more important for the AG-CP and the Shared gamma frailty models despite their power (ability to detect significant effect) than in the PWP-CP and GEE Poisson distribution models. But, according to the empirical coverage rate as described by Burton *et al.* [31], the PWP-CP remained the more robust to the addition of the simulated confounding factor.

Conclusions

In the context of malaria, statistical models have to take recurrent events and discontinuous time into account to better estimate effects of covariates such as treatments or risk factors such as age on malaria recurrent episodes. In this context, the three extended Cox models presented here are of high interest and showed similar results.

Additional file

Additional file 1: Stata (Stata Corporation 2011) and R codes for the different models.

Competing interests

The authors do not have any competing interests to declare.

Authors' contributions

IS conceived the study, prepared the data, carried out the data analysis, wrote an initial draft of the manuscript, and worked on the production of final draft. RG helped in the study, advised on data analysis, and contributed to the writing of the manuscript. OKD contributed to the interpretation of the results and the writing of the manuscript. RP contributed to the interpretation of the results and the writing of the manuscript. JG helped in the study conception, advised on the data preparation, contributed to the data analysis, and worked on the production of final draft. All authors read and approved the final manuscript.

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Additional file 1 Stata (Stata Corporation 2011) and R codes for the different models

Models	Stata codes	R codes
GEE Poisson distribution	xi: xtgee episode i.treatcat i.agecat, eform f(poisson) i(ID) l(log) cor(exch) t(time) robust	mod_gee <- gee(episode~ treatcat + agecat, data = table_long, id = ID, family = poisson, corstr = "exchangeable")
AG-CP	xi: stcox i.treatcat i.agecat, efron nolog cluster(ID)	mod_AG<-coxph(Surv(start, end, episode)~treatcat+agecat+ cluster="ID", data= table_long)
PWP-CP	xi: stcox i.treatcat i.agecat, efron nolog cluster(ID) strata(order)	mod_PWP<- coxph(Surv(start, end, episode)~treatcat+agecat+ cluster="ID", strata="order",data= table_long)
Frailty Gamma	xi: streg i.treatcat i.agecat, d(weib) frailty(gamma) shared(ID) nolog	mod_frailty<- coxph(Surv(start, end, episode)~treatcat+agecat+ frailty (ID, dist="gamma"),data= table_long)

Variables names and signification are provided in the data dictionary in Table 1.

For Stata codes:

For GEE Poisson distribution: xi: meaning using dummy codes for independent variables (i.treatcat=treatment category, i.agecat=age. Dummy codes for treatment category were: AL=0, AS+AQ=1, AS+SP=2; Dummy codes for age category were: <5year=2, 5-9years=1, >9years=0; xtgee function fits a population-averaged panel-data models by using GEE; eform is the displaying function for the exponentiated coefficients; f(poisson) is poisson family function; i is the patient identification variable; l(log) is logarithm link function; cor(exch) is the correlation structure function with exchangeable option; t(time) is the time function linking the time/duration (days here); robust is a roust standard error function.

For AG-CP, PWP-CP and Frailty Gamma models, there is a need to declare the survival expression by the following syntax: stset end, failure(episode) exit(end) enter(start).

For R codes: require gee and survival packages.

For GEE Poisson distribution: mod_gee is the object name; gee is a function for generalized estimation equation; table_long is the data format used in Table 1; id is the patient identification variable; corstr is the correlation structure.

For AG-CP and PWP-CP models: mod_AG and mod_PWP are the object name respectively for AG and PWP-CP models; coxph is the Cox Proportional hazard function; Surv is the Survival function; strata fits a stratified Cox model by ordered malaria episodes.
For Frailty Gamma model: mod_frailty is the object name; frailty is the shared frailty function within patient with gamma distribution.

IV. Agrégation d'essais cliniques pour l'évaluation d'effets indésirables : cas de l'anémie post-thérapeutique

La méta-analyse classique consiste à analyser les résultats des études cliniques précédentes avec pour objectif d'évaluer la magnitude de l'effet d'une exposition avec une plus grande puissance [12], mais peut introduire une grande variabilité due à l'hétérogénéité méthodologique potentielle entre les différents protocoles d'études [13]. Tout en ayant le même objectif que celui de la méta-analyse, l'analyse après l'agrégation des données individuelles issues des différents essais cliniques permet une plus grande standardisation des procédures méthodologiques, donc une réduction d'hétérogénéité entre les études cliniques et aussi permet parfois d'étudier d'autres covariables non rapportés dans les resultats d'études initiales [14-15].

L'anémie post-thérapeutique d'épisode de paludisme avec les dérivés d'artémisinine est un effet indésirable qui fait l'objet de débats actuellement [16]. En effet, les cas rapportés d'anémie retardée dans la littérature après un traitement de paludisme avec les dérivés d'artémisinine étaient des études de cas ou de séries de cas [17-22].

Pour répondre à la question de l'anémie post-thérapeutique d'accès palustre avec les dérivés d'artémisinine, nous avons agrégé des données individuelles de 13 études cliniques distinctes et utilisé d'outils statistiques appropriés pour l'analyse de données (le taux d'hémoglobine qui était la variable réponse d'intérêt était mesuré répétitivement mais variable selon le protocole d'étude au jour 0, 3, 7, 14 et 28). Les analyses multivariées ont utilisé des modèles linéaires et latents généralisés mixtes (« GLLAMM : generalized linear and latent mixed model »). Les données provenaient de 13 essais cliniques de traitement antipaludique conduits entre 2002 et 2011 dans les différentes régions du Mali. Les différents médicaments antipaludiques étaient : l'artéméther-luméfantrine (AL), l'artésunate/amodiaquine (AS-AQ), l'artésunate/ sulfadoxine-pyrimethamine (AS-SP), l'artésunate/sulfaméthoxypyrazine-

pyriméthamine (AS-SMP), l'artésunate/ méfloquine (AS-MEF), l'artésunate-pyronaridine (ASPYR), l'artésunate monothérapie (AS), la chloroquine (CQ), la sulfadoxine-pyriméthamine (SP), l'amodiaquine (AQ) et la sulfadoxine-pyriméthamine/ amodiaquine (SP+AQ).

Le GLLAMM appartient à la classe des modèles à multinivaux ou hiérarchiques. L'approche mixte permet la modélisation des covariables à effets aussi bien fixes qu'aléatoires et ainsi de prendre en compte, la part de variabilité introduite par l'agrégation d'études différentes. La variable latente est soit un facteur fixe, soit un effet aléatoire, ou soit une nuisance. Plusieurs publications utilisant ou discutant les procédures d'analyse GLLAMM sont disponibles [23]. Dans cette présente analyse, les covariables à effets fixes étaient l'âge des participants, la densité parasitaire initiale (log transformée), le traitement antipaludique, et l'échec au traitement antipaludique pendant le suivi. Le site était utilisé comme variable aléatoire ou latente. La variable dépendante utilisée était soit le taux d'hémoglobine en variable continue soit le taux d'hémoglobine transformé en variable dichotomique ; l'anémie (taux d'hémoglobine <10g/dl pour l'anémie, taux d'hémoglobine <8g/dl pour l'anémie sévère) soit la chute du taux d'hémoglobine à un seuil de 1 ou 2 g/dl.

Les résultats de cette analyse dont les travaux sont publiés en 2014 dans « *Malaria Journal*» dont l'article est joint ci-dessous n'ont pas mis en évidence d'association entre l'anémie sévère retardée et les traitements à base d'artémisinine. Cependant, les dérivés de l'artémisinine utilisés pour le traitement du paludisme *P. falciparum* étaient associés à une baisse transitoire et cliniquement modérée du taux d'hémoglobine au jour 7, ce que confirment les études précédentes [24-27].

Article 3: «Delayed anemia assessment in patients treated with oral artemisinin derivatives for uncomplicated malaria: a pooled analysis of clinical trials data from Mali»

MALARIA JOURNAL

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Delayed anemia assessment in patients treated with oral artemisinin derivatives for uncomplicated malaria: a pooled analysis of clinical trials data from Mali

Issaka Sagara[1,2*], Renaud Piarroux[3], Abdoulaye Djimde[1], Roch Giorgi[2], Kassoum Kayentao[1], Ogobara K Doumbo[1] and Jean Gaudart[2]

Abstract

Background: In sub-Saharan Africa, artemisinin-based combination therapy (ACT) and injectable artesunate are the first-line treatments for uncomplicated and severe *Plasmodium falciparum* malaria, respectively. However, recent studies suggest that delayed anaemia is associated with these treatments in non-immune travellers. This paper aimed to assess the risk factors associated with delayed anaemia after falciparum malaria treatment with artemisinin-containing drugs in malaria-endemic populations.

Methods: Pooled, individual malaria patient data were extracted from 13 clinical trials performed from 2002 to 2011 in various settings of Mali. Treatment regimens were artemether-lumefantrine, artesunate plus amodiaquine, artesunate plus sulphadoxine-pyrimethamine, artesunate plus sulphamethoxypyrazine-pyrimethamine, artesunate plus mefloquine, artesunate-pyronaridine, artesunate monotherapy, chloroquine, sulphadoxine-pyrimethamine, amodiaquine and sulphadoxine-pyrimethamine plus amodiaquine. Univariate and multivariate analyses were performed using the generalized linear and latent mixed model procedures to assess risk factors associated with haemoglobin concentration evolution and anaemia during the treatment follow-up.

Results: A total of 5,990 participants were recruited and followed from day 0 to day 28. The participants' median age was five years, ranging from three months to 70 years. There was a decrease in haemoglobin level on day 7 in all treatment arms, but the magnitude varied across treatments. There was a significant risk of haemoglobin level decrease on day 7 in the artemisinin-based therapy compared to the non-artemisinin treatments. The risk of haemoglobin concentration drop was associated with age group < five years old (0.61 g/dL 95% CI (0.71 to 0.51), $p < 0.001$), baseline high parasite density (0.43 g/dL 95% CI (0.51 to 0.35), $p < 0.001$) and treatment failure (0.40 g/dL 95% CI (0.59 to 0.20), $p = 0.018$), while high haemoglobin level at baseline was a protective factor (0.53 to 0.59) $p < 0.001$). No association was found between artemisinin-based therapy and severe delayed anaemia.

Conclusions: Oral artemisinin derivative treatments for uncomplicated *P. falciparum* malaria are associated with a transient and clinically moderate haemoglobin decrease by day 7 but not associated with a delayed severe anaemia.

Keywords: Uncomplicated, *Plasmodium falciparum*, Malaria, Delayed anaemia, Artemisinin, Clinical trials

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Background

According to the World Health Organization (WHO) 2013 report, 207 million cases of malaria were estimated to have occurred in 2012 with 627,000 deaths [1]. Early diagnosis and treatment of malaria reduce disease progression, prevent deaths and contribute to reduce malaria transmission [2]. To counter the threat of resistance of *Plasmodium falciparum* to monotherapy, and to improve treatment outcome, WHO recommends the use of artemisinin-based combination therapy (ACT) for the treatment of uncomplicated *P. falciparum* malaria [3], and the injectable artesunate as the first-line treatment of severe malaria [4]. However, recent data, mainly from case reports raised concerns about possible delayed anaemia associated with artemisinin derivative treatments [5-9]. WHO recognized that post-treatment haemolytic anaemia was not specific to a particular injectable artesunate formulation and that anaemia was also described following the use of intramuscular artemether and oral artemether-lumefantrine [10,11]. Therefore, WHO called for the systematic monitoring of haemoglobin after malaria treatment initiation up to one month.

There is limited large-scale data in the literature from malaria-endemic areas assessing haematological parameters, particularly anaemia among patients treated with the artemisinin-containing malaria treatments. Recently a publication from a large-scale, pooled, multi-country study data from malaria-endemic areas assessed haematologic parameter changes in patients treated with ACT (artesunate-amodiaquine, artesunate plus sulphadoxine-pyrimethamine, dihydro-artemisinin-piperaquine and artemether-lumefantrine) and other therapy (artesunate, amodiaquine and sulphadoxine-pyrimethamine plus amodiaquine) [12]. That study found more anaemia adverse events in the artemisinin-based therapy compared to the non-artemisinin therapy at the 28th day of follow-up, but did not provide the specific day of the occurrence of anaemia during the follow-up period. As precise knowledge of the specific occurrence day of haemoglobin change or anaemia occurrence is of clinical relevance, this paper aimed to perform a pooled analysis of data from 13 clinical trials conducted in Mali. The goal of this pooled data analysis was to describe the dynamics of haemoglobin levels, delayed anaemia and the associated risk or protective factors during each follow-up day, up to 28 days.

Methods

Data source

A pooled, malaria-treatment, clinical trial analysis was performed, using individual data extracted from clinical trial databases of 13 trials conducted by researchers from Malaria Research and Training Centre (MRTC), Bamako, a research institution within the University of Sciences, Techniques and Technologies of Bamako (USTTB), Mali. The different studies were performed from 2002 to 2011 in various malaria-transmission settings of Mali [13-23] (Ouologuem D *et al.* Host immunological factors involved in clearance of drug resistant falciparum malaria, unpublished observation; Maiga H *et al.* Dhfr-dhps quadruple mutant predicts sulfadoxine-pyrimethamine resistance in Mali, an emerging sulfadoxine-pyrimethamine resistance setting, unpublished observation).

Treatment regimens

The different malaria treatment regimens were: (i) ACT: artemether-lumefantrine ((AL) Coartem® from Novartis); artesunate plus amodiaquine ((AS-AQ), loose or fixed-dose combination, Arsucam/Coarsucam®, both from Sanofi-Aventis); artesunate plus sulphadoxine-pyrimethamine ((AS-SP), loose combination; AS, Arsumax® from Sanofi-Aventis and SP, Fansidar® from Roche); artesunate plus sulphamethoxypyrazine-pyrimethamine ((AS-SMP), loose combination, Coarinate® from Dafra Pharma); artesunate plus mefloquine ((AS-MEF), loose combination, Artequin® from Mepha Pharma); and, artesunate-pyronaridine ((AS-PYR), fixed-dose combination, Pyramax® from Shin Poong); (ii) artesunate oral monotherapy ((AS), Arsumax® or Asunate Denk® respectively from Sanofi-Aventis and Denk Pharma); (iii) non ACT: chloroquine (CQ); amodiaquine (AQ); sulphadoxine-pyrimethamine (SP) and sulpha doxine-pyrimethamine plus amodiaquine ((SP-AQ), loose combination).

Treatment courses durations were three days for all except for: a) SP given as a single dose; and, b) AS given up to five days in one study [21] and seven days in another study [13]. The treatment doses were given by the study investigators and details are provided in each study reference [13-23] (Ouologuem D *et al.* Host immunological factors involved in clearance of drug resistant falciparum malaria, unpublished observation; Maiga H *et al.*: Dhfr-dhps quadruple mutant predicts sulfadoxine-pyrimethamine resistance in Mali, an emerging sulfadoxine-pyrimethamine resistance setting, unpublished observation) and also in a Additional file 1.

Outcome definitions

Data were systematically analysed according to three outcomes: dynamic of haemoglobin level, haemoglobin drop and anaemia occurrence.

Haemoglobin levels were analysed as a continuous variable (haemoglobin changes outcome) and as categorical variables (haemoglobin drop and anaemia occurrence). Drop of haemoglobin value was defined from day 0 to the day of the different follow-up visit (day 7, 14, 28) with the following thresholds: ≥1 g/dL or ≥2 g/dL. Anaemia was defined as a haemoglobin value <10 g/dL and severe anaemia as haemoglobin value <8 g/dL as described elsewhere [12]. Delayed anemia was defined as an anaemia

(severe or not) observed any time of follow-up from day 7 to day 28 [5].

For these outcomes, multivariate analyses assessed the different associated risk factors during each visit after treatment (day 7, 14 or 28) in order to determine any significant changes during the follow-up. These analyses included all participants for whom haemoglobin data were available on day 0 and at least one follow-up day (day 7 or 14 or 28). Specifically for the delayed severe anaemia occurrence, associated risk factors were assessed with different time points: (i) from day 7 to day 28; (ii) from day 14 to day 28; and, (iii) on day 28. These analyses used a subset of participants for which the haemoglobin data were available for all follow-up visits (day 0, 7, 14, and 28). Different late or delayed severe anaemia periods were assessed: (i) an absence of severe anaemia on day 0 followed by its occurrence during any subsequent days for follow-up (7 or 14 or 28); (ii) an absence of severe anaemia on day 0 and day 7 followed by its occurrence during any subsequent days for follow-up (i.e., day 14 or 28); and, (iii) an absence of severe anaemia on day 0 and day 7 and day 14 followed by its occurrence on day 28.

Statistical analysis

Descriptive statistics provided the total number of participants, means, standard deviations, medians, minimums, and maximums for quantitative variables and proportions for qualitative variables.

Univariate analyses assessed the haemoglobin changes between day 0 and the day of post-treatment evaluation (day 7, 14 or 28) using paired Student test in each treatment arm.

For both continuous variable of haemoglobin changes and categorized variable of haemoglobin drop or anaemia outcomes, multivariate analyses were performed using generalized linear and latent mixed model (GLLAMM), with study site as a latent factor and as random effect. Models were systematically adjusted for covariates such as age, parasite density at baseline (log transformed), malaria treatment, and treatment failure during the follow-up. For the haemoglobin changes outcome, the gaussian family (with the identity canonical link) was used to estimate the coefficients of covariate effects. For the categorized haemoglobin drop and anaemia outcomes, the binomial family (the logit canonical link) was used to estimate odds ratios associated with covariates.

The confidence intervals (CIs) were estimated at 95% and p value <0.05 was considered significant. Data were analysed with GLLAMM procedure using Stata Software version 12.1 (Stata Corp).

Ethical issues

Each study had a prior approval from the institutional ethical committee at the Faculty of Medicine, Pharmacy and Odonto-Stomatogy (FMPOS)/USTTB, Bamako, Mali. Written consent was obtained in each study from each participant or parent/legal guardian.

Results

Study information and baseline characteristics

This pooled data of 13 trials in eight Malian sites (Figure 1) enrolled 5,990 participants with various malaria treatments (Table 1), including ACT (AL, AS-AQ, AS-SP, AS-SMP, AS-MEF, AS-PYR; oral AS monotherapy; and, non ACT (CQ, AQ , SP and SP-AQ).

Table 2 shows that the median age for the pooled data was five years, ranging from three months to 70 years. The baseline (day 0) proportion of anaemia was 36% and the baseline parasite density geometric mean was 17,789 parasites/μl.

Assessment of haemoglobin level per visit day

On day 7: overall, there was an average haemoglobin decrease of 0.4 g/dL compared to day 0. Univariate analyses showed a significant decrease ($p < 0.001$) in all treatments except for AQ (Table 3). The frequency of haemoglobin drop to ≥1 g/dL and to ≥2 g/dL compared to day 0 was 32.2% (858/2,668) and 11.4% (303/2,668), respectively.

Multivariate analyses showed a higher risk for haemoglobin decrease in the ACT (AS or the combined AS-AQ/AS-SP/AS-PYR and AL) compared to the non ACT (CQ/AQ/SP). The risk of haemoglobin decrease was significant for the AS, mean decrease of 0.69 g/dL 95% CI (0.95-0.44), $p < 0.001$; and the AS-containing combinations: AS-AQ/AS-SP/AS-PYR, 0.34 g/dL, 95% CI (0.57-0.11), $p = 0.003$ (Table 4). A similar trend was observed for the haemoglobin drop to ≥1 g/dL for AS and the AS-containing combinations: AS-AQ/AS-SP/AS-PYR, but was only significant for the AS arm (OR = 1.69, 95%CI (1.31-2.18); $p < 0.001$) (Table 5). There was no risk associated with a haemoglobin drop ≥2 g/dL on day 7 with artemisinin-derivative treatments (Table 6). There was even a protective effect against haemoglobin drop ≥2 g/dL with AL (OR = 0.51, 95% CI (0.28-0.90); $p = 0.02$) and the AS-containing combinations: AS-AQ/AS-SP/AS-PYR (OR = 0.70, 95% CI (0.52-0.94); $p = 0.02$) compared to non-artemisinin treatments (Table 6).

A significant risk of haemoglobin decrease was associated with other covariates (Table 4) such as the age group < five years ($p < 0.001$), a high parasite density at baseline ($p < 0.001$), a treatment failure ($p = 0.018$) and a lower baseline haemoglobin level ($p < 0.001$). There was also a significant risk of haemoglobin drop ≥1 g/dL and ≥2 g/dL for patients showing a high parasite density at baseline with OR of 1.56 (95% CI (1.33-1.83); $p < 0.001$) and 1.80 ((1.41-2.30); $p < 0.001$), respectively (Tables 5 and 6).

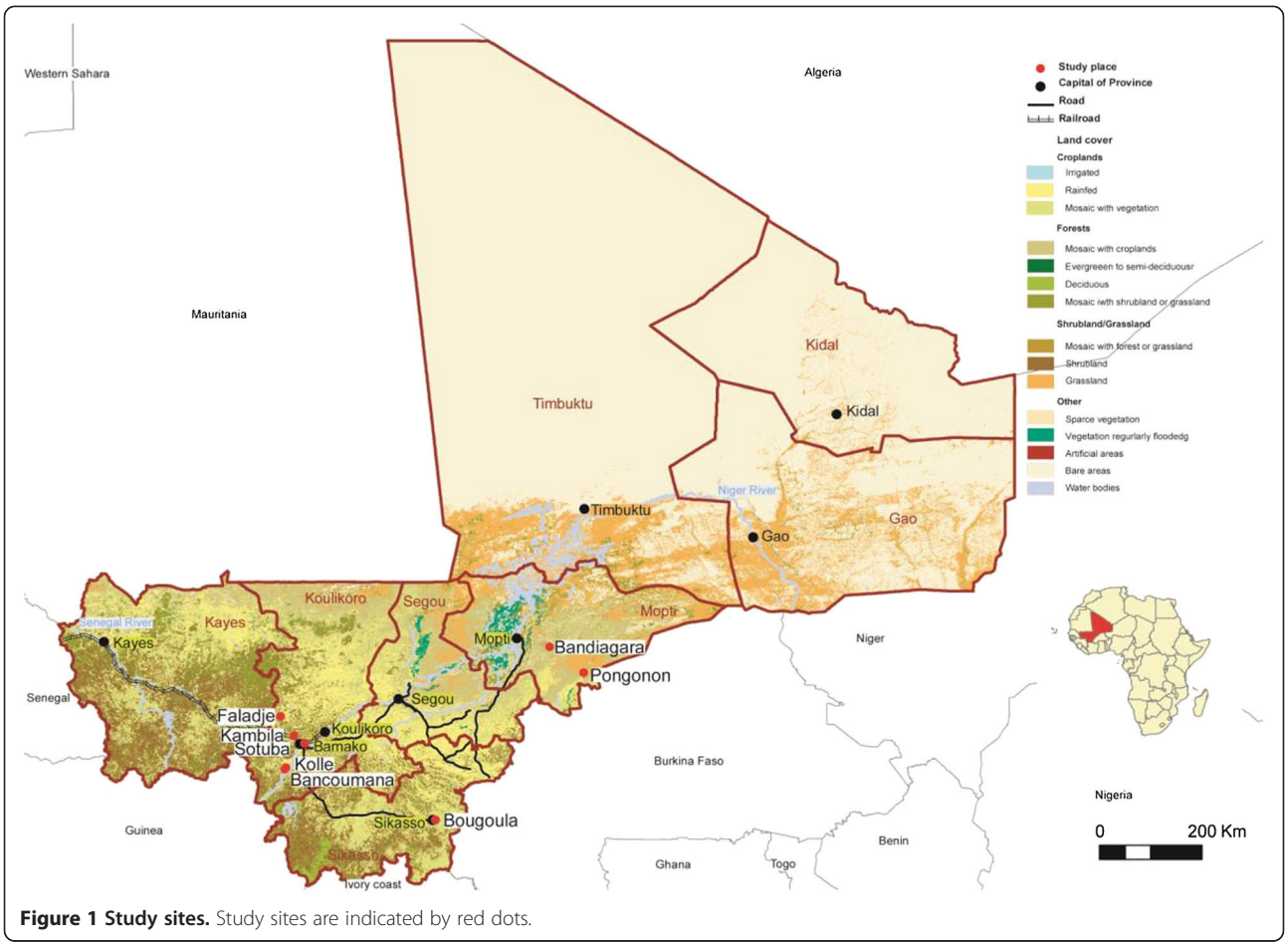

Figure 1 Study sites. Study sites are indicated by red dots.

Table 1 Number of patients per study and treatment arm

Study* (year)	Treatment											
	AL	AS-AQ	AS-PY	AS-SP	AS-SMP	AS-MF	AS	SPAQ	SP	AQ	CQ	Total
[13] (2010–11)							100					100
UO (2009)	337											337
[14] (2007–08)	44		86									130
[15] (2007)	66		132									198
[16] (2006)	68	135										203
[17] (2005–07)	257	255		259								771
[18] (2005)		133		134				133				400
[19] (2004–05)	235					235						470
[20] (2003–04)	303				303							606
[21] (2002–04)		252		249			251					752
UO (2002–04)											948	948
UO (2002–03)									619			619
[22] (2002–03)									121	115	220	456
Total	1,310	775	218	642	303	235	351	133	740	115	1,168	5,990

*The studies are cited in descending order from conduct year ranging from 2011 to 2002. Numbers in the first column represent publication references as cited in the text. UO, unpublished observations.

Table 2 Baseline characteristics and scheduled hemoglobin evaluation visit per study and site

Study*	Site	N	Haemogobin evaluation days	Age median [min, max]	*P. f.*	Anaemia prev.
[13] (2010–11)	Bougoula- Hameau	100	0, 3, 14, 28	6 [1,11]	28,027	48%
UO (2009)	Kolle	77	0, 14, 28	6 [1,18]	44,492	55%
	Faladje	88		6 [1,14]	47,942	26%
	Bandiagara	100		7 [1, 61]	32,703	28%
	Pongonon	72		5 [1, 34]	18,841	43%
[14] (2007–08)	Bougoula- Hameau	130	0, 3, 7, 28	6 [2,10]	32,430	31%
[15] (2007)	Bougoula- Hameau	198	0, 3, 7, 28	11 [6, 52]	22,115	11%
[16] (2006)	Bancoumana	203	0, 7, 14, 28	5 [0.9, 24]	22,183	50%
[17] (2005–07)	Bougoula- Hameau	771	0, 7, 14, 28	4 [0.5, 69]	20,463	50%
[18] (2005)	Faladje	400	0, 14, 28	36 [6, 60]	23,705	48%
[19] (2004–05)	Kambila	470	0, 14, 28	6 [1,70]	18,258	13%
[20] (2003–04)	Sotuba	606	0, 28	11 [0.6, 63]	13,592	14%
[21] (2002–04)	Bougoula- Hameau	752	0, 3, 7, 14, 28	3 [0.6, 56]	15463	44%
UO (2002–04)	Kolle	655	0, 7, 14	7 [0.5, 64]	12200	25%
	Bandiagara	293		6 [0.7, 30]	12686	29%
UO (2002–03)	Bougoula- Hameau	619	0, 14, 28	3 [0.5, 65]	14897	42%
[22] (2002–03)	Kolle	456	0, 7, 14, 28	3 [0.3, 6]	19778	51%
Total		5,990		5 [0.3, 70]	17,789	36%

Note: *P. f., Plasmodium falciparum* geometric mean; Anaemia prev. (dichotomous variable), anaemia prevalence. Anaemia was defined as haemoglobin value <10.0 g/dL; Numbers in first column represent publication references as cited in the text.
*The studies are citied in descending order from conduct year ranging from 2011 to 2002. The number given is their publication reference as applicable.
UO, unpublished observations.

On day 14: overall, there was an average haemoglobin increase of 0.13 g/dL compared to day 0. Univariate analyses in Table 3 showed a relatively small increase of haemoglobin level on day 14 compared to day 0 in all treatment arms except for AS-MEF. The frequency of haemoglobin drop ≥1 g/dL and ≥2 g/dL compared to day 0 was 21.6% (1,004/4,648) and 8% (371/4,648), respectively.

The multivariate analysis showed no risk of haemoglobin decrease or haemoglobin drop ≥1 g/dL, or haemoglobin drop ≥2 g/dL associated with any of the malaria treatment arms on day 14 (Tables 4, 5 and 6). However, there was a

Table 3 Haemoglobin changes from baseline to day of follow-up after treatment per treatment arm

	Day 0-day 7				Day 0-day 14				Day 0-day 28			
	N	Mean 0 [SD]	Mean 7 [SD]	Mean diff, (P-value)	N	Mean 0, [SD]	Mean 14, [SD]	Mean diff, P-value	N	Mean 0, [SD]	Mean 28, [SD]	Mean diff, (P-value)
AL	193	10.6 [1.7]	10.2 [1.7]	−0.4 **(<0.001)**	861	10.5 [1.9]	10.7 [1.6]	+0.2 **(0.015)**	890	10.7 [1.8]	11.3 [1.6]	+0.6 **(<0.001)**
AS-AQ	403	10.1 [1.8]	9.7 [1.7]	−0.4 **(<0.001)**	729	10.0 [1.7]	10.2 [1.5]	+0.2 **(<0.001)**	667	10.0 [1.8]	10.7 [1.7]	+0.7 **(<0.001)**
AS-PYR	218	11.4 [1.5]	11.0 [1.5]	−0.4 **(0.001)**	–	–	–	–	213	11.4 [1.5]	11.9 [1.3]	+0.4 **(<0.001)**
AS-SP	272	10.2 [1.9]	9.7 [1.7]	−0.5 **(<0.001)**	630	10.0 [1.8]	10.1 [1.6]	+0.1 **(0.023)**	610	10.0 [1.8]	10.7 [1.5]	+0.7 **(<0.001)**
AS-SMP	–	–	–	–	–	–	–	–	105	11.8 [1.8]	12.0 [1.5]	+0.2 (0.096)
AS-MEF	–	–	–	–	228	11.4 [1.5]	11.2 [1.3]	−0.2 **0.032**	213	11.5 [1.5]	11.9 [1.1]	+0.3 **(0.0003)**
AS	348	10.2 [1.7]	9.5 [1.5]	−0.7 **(<0.001)**	348	10.2 [1.7]	10.3 [1.4]	+0.1 (0.198)	297	10.2 [1.8]	11.0 [1.5]	+0.8 **(<0.001)**
SP-AQ	–	–	–	–	133	10.0 [1.6]	10.4 [1.5]	+0.4 **(0.001)**	131	10.0 [1.6]	11.1 [1.5]	+1.1 **(<0.001)**
SP	114	10.0 [1.8]	9.4 [1.5]	−0.6 **(<0.001)**	679	10.2 [1.8]	10.3 [1.7]	+0.1 (0.102)	706	10.3 [1.8]	10.9 [1.7]	+0.6 **(<0.001)**
AQ	104	9.9 [1.5]	9.7 [1.5]	−0.2 (0.3)	62	9.7 [1.6]	9.9 [1.3]	+0.2 (0.251)	97	9.9 [1.5]	10.4 [1.6]	+0.5 **(0.002)**
CQ	1016	10.9 [1.9]	10.6 [1.7]	−0.3 **(<0.001)**	978	10.9 [1.9]	11.0 [1.7]	+0.1 (0.065)	162	9.86 [1.63]	10.01 [1.54]	+0.2 (0.261)

Note: Mean 0, Mean 7, Mean 14, Mean 28 are haemoglobin mean on days 0, 7, 14, and 28, respectively; SD, standard deviation of the mean;
Mean diff, haemoglobin mean on the day of the follow-up minus haemoglobin mean on day 0.

Table 4 Multivariate analysis assessing haemoglobin changes per treatment arm during different post-treatment follow-up visits

	Day 7 (n=2,657)		Day 14 (n=4,638)		Day 28 (n=4,082)	
Covariates**	**β* (95% CI)**	***p***	**β* (95% CI)**	***p***	**β* (95% CI)**	***p***
AL	−0.21 (−0.48-0.05)	*0.11*	−0.06 (−0.17- 0.06)	*0.34*	−0.05 (−0.18- 0.08)	*0.47*
AS+partners	−0.34 (−0.57- -0.11)	***0.003***	−0.12 (−0.22- -0.02)	***0.02***	−0.03 (−0.14- 0.08)	*0.62*
AS	−0.69 (−0.95- -0.44)	***<0.001***	−0.03 (−0.18-0.11)	*0.68*	0.15 (−0.02-0.32)	*0.08*
<5 years old	−0.61 (−0.71- -0.51)	***<0.001***	−0.62 (−0.71- -0.54)	***<0.001***	−0.51 (−0.60- -0.42)	***<0.001***
Haemo day 0	0.56 (0.53-0.59)	***<0.001***	0.48 (0.46-0.50)	***<0.001***	0.45 (0.43-0.47)	***<0.001***
P. f. day 0	−0.43 (−0.51- -0.35)	***<0.001***	−0.33 (−0.40- -0.026)	***<0.001***	−0.04 (−0.12-0.04)	*0.29*
Treat fail	−0.40 (−0.59- -0.20)	***0.018***	−0.25 (−0.39- -0.11)	***<0.001***	−0.4 (−0.51- -0.29)	***<0.001***

Note: *β is the regression coefficient estimate obtained from the generalized linear latent and mixed models using site as random effect;
**Covariates references were: CQ, AQ, SP for day 7 and CQ, AQ, SP and SP-AQ for day 14 and day 28 for treatment, the age ≥5 years old for the age group, the 28-day cured parasite outcome after treatment for the treatment failure (Treat fail) outcome, the baseline parasite density was log transformed and treated as continuous variable; AS + partners were: AS-AQ, AS-SP, AS-PYR for day 7, AS-AQ, AS-SP, AS-MEF for day 14 and AS-AQ, AS-SP, AS-PYR, AS-SMP and AS-MEF for day 28.

significant risk of haemoglobin decrease or drop to ≥1 g/dL or to ≥2 g/dL with a high parasite density at baseline and the treatment failure (Tables 4, 5 and 6).

On day 28: overall, there was an average haemoglobin increase of 0.63 g/dL compared to day 0. Univariate analyses in Table 3 indicate that the increases were significant in all treatment arms except for CQ (p = 0.261) and AS-SMP (p = 0.096). The frequency of haemoglobin drop ≥1 g/dL and ≥2 g/dL compared to day 0 was 13.9% (569/4,091) and 4.9% (200/4,091), respectively.

Multivariate analyses showed no risk of haemoglobin decrease or haemoglobin drop ≥1 g/dL or haemoglobin drop ≥2 g/dL associated with any malaria treatment arms on day 28 (Tables 4, 5 and 6). However, there was a significant risk of haemoglobin decrease or drop ≥1 or ≥2 g/dL with treatment failure (Tables 4, 5 and 6), with the higher risk association for haemoglobin drop ≥2 g/dL (OR: 2.03, 95% CI (1.44-2.87), p < 0.001) (Table 6).

Assessment of anaemia and severe anaemia

Anaemia (haemoglobin <10 g/dL) frequency on day 0, 7, 14, and 28 was 35.5% (2,115/5,957), 45.7% (1,221/2,672), 35.3% (1,640/4,652), and 23.9% (979/4,099), respectively.

The severe anaemia (haemoglobin <8 g/dL) frequency on day 0, 7, 14, and 28 was, respectively, 9.0% (536/5,957), 10.1% (269/2,672), 6.2% (286/4,652), and 4.0% (165/4,099).

Among a total of 1,053 eligible patients (with no severe anaemia on day 0 and haemoglobin data available on days 0, 7, 14, and 28), 135 patients (12.8%) experienced severe anaemia from day 7 to day 28. Multivariate analysis showed a risk of severe anaemia associated with the AS arm from day 7 to day 28 (OR = 1.85 (1.05-3.27); p = 0.03) compared to the non-artemisinin-contained treatments (CQ alone/AQ alone/SP alone/SP-AQ) (Figure 2). But, no risk was present with the other artemisinin-derivative treatments (AL, AS-AQ/AS-SP).

Table 5 Multivariate analysis estimating risk of haemoglobin drop from baseline to ≥1 g/dL during different post-treatment follow-up visits

	Day 7 (2,657)		Day 14 (n=4,638)		Day 28 (n=4,082)	
	OR* (95% CI)	***p***	**OR* (95% CI)**	***p***	**OR* (95% CI)**	***p***
Covariates**						
AL	0.82 (0.58-1.17)	*0.27*	1.03 (0.82-1.30)	*0.81*	1.10 (0.82-1.48)	*0.52*
AS+partners	1.15 (0.95-1.39)	*0.16*	0.93 (0.75-1.14)	*0.47*	1.0 (0.77-1.29)	*0.99*
AS	1.69 (1.31-2.18)	***<0.001***	0.97 (0.71-1.32)	*0.84*	1.01 (0.68-1.50)	*0.95*
<5 years old	0.90 (0.76-1.06)	*0.21*	0.79 (0.68-0.92)	***0.003***	0.72 (0.60-0.88)	***0.001***
P. f. day 0	1.56 (1.33-1.83	***<0.001***	1.60 (1.38-1.87)	***<0.001***	1.02 (0.85-1.23)	*0.81*
Treat fail	1.77 (1.24-2.55)	***0.002***	1.49 (1.14-1.94)	***0.003***	1.67 (1.32-2.11)	***<0.001***

Note: *OR is the odds ratio obtained from generalized linear and latent mixed model with binomial family using site as random effect;
**Covariates references were: CQ, AQ, SP for day 7 and CQ, AQ, SP and SP-AQ for day 14 and day 28 for treatment, the age ≥5 years old for the age group, the 28-day cured parasite outcome after treatment for the treatment failure (Treat fail) outcome, the baseline parasite density was log transformed and treated as continuous variable; AS + partners were: AS-AQ, AS-SP, AS-PYR for day 7, AS-AQ, AS-SP, AS-MEF for day 14 and AS-AQ, AS-SP, AS-PYR, AS-SMP and AS-MEF for day 28.

Table 6 Multivariate analysis estimating risk of haemoglobin drop from baseline to ≥2 g/dL during different post-treatment follow-up visits

	Day 7 (2,657)		Day 14 (n=4,638)		Day 28 (n=4,082)	
	OR* (95% CI)	*p*	OR*(95% CI)	*p*	OR* (95% CI)	*p*
Covariates**						
AL	0.51 (0.28-0.90)	***0.02***	1.33 (0.95-1.87)	*0.10*	0.86 (0.54-1.39)	*0.55*
AS+partners	0.70 (0.52-0.94)	***0.02***	0.95 (0.69-1.32)	*0.78*	0.96 (0.63-1.44)	*0.83*
AS	1.36 (0.97-1.92)	*0.08*	0.99 (0.61-1.59)	*0.96*	1.07 (0.58-1.96)	*0.83*
<5 years old	0.87 (0.68-1.11)	*0.25*	0.82 (0.65-1.04)	*0.10*	0.82 (0.60-1.12)	*0.21*
P.f. day 0	1.80 (1.41-2.30	***<0.001***	1.68 (1.33-2.12)	***<0.001***	1.30 (0.96-.75)	*0.09*
Treat fail	1.43 (0.88-2.32)	*0.15*	1.63 (1.13-2.38)	***0.01***	2.03 (1.44-2.87)	***<0.001***

Note: *OR is the odds ratio obtained from generalized linear and latent mixed model with binomial family using site as random effect;
**Covariates references were: CQ, AQ, SP for day 7 and CQ, AQ, SP and SP-AQ for day 14 and day 28 for treatment, the age ≥ 5 years old for the age group, the 28-day cured parasite outcome after treatment for the treatment failure (Treat fail) outcome, the baseline parasite density was log transformed and treated as continuous variable; AS + partners were: AS-AQ, AS-SP, AS-PYR for day 7, AS-AQ, AS-SP, AS-MEF for day 14 and AS-AQ, AS-SP, AS-PYR, AS-SMP and AS-MEF for day 28.

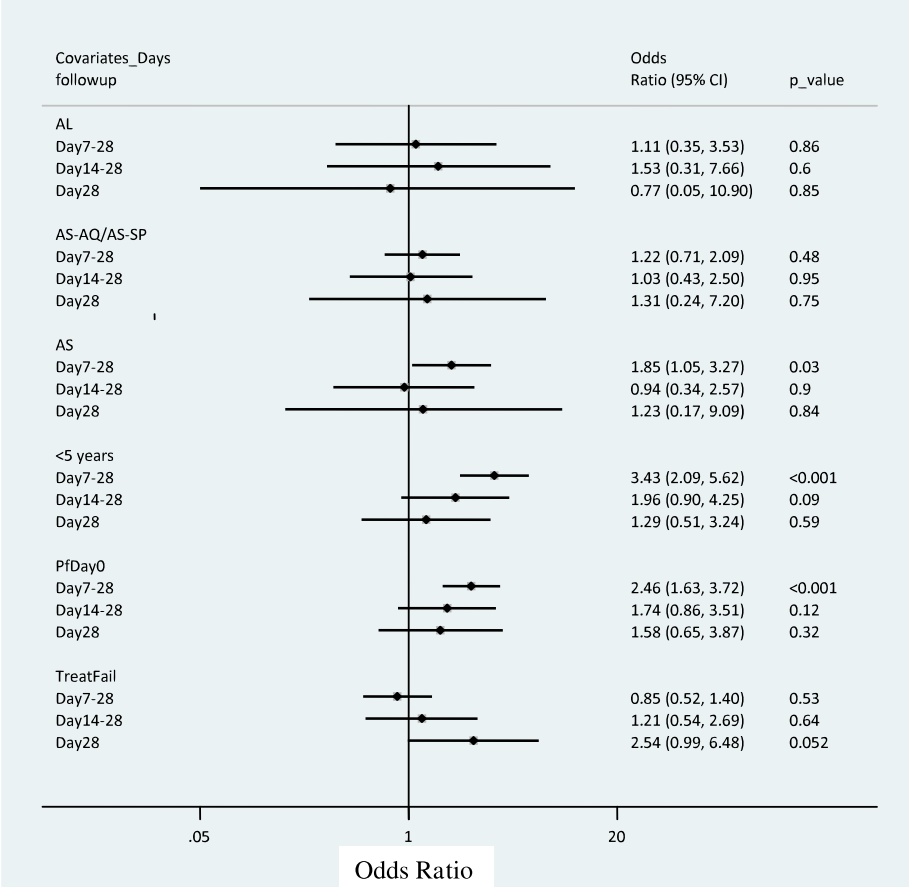

Figure 2 Multivariate analysis estimating late severe anaemia occurrence risk among patients who participated to all haemoglobin evaluation from day 0, 7, 14, and 28. n = 1,053, 956 and 941, respectively, for patients who participated to severe anaemia evaluation from day 7 to day 28, day 14 to day 28, and day 28. AL, artemether-lumefantrine; AS-AQ/AS-SP, artesunate plus amodiaquine/artesunate plus sulphadoxine-pyrimethamine; AS, artesunate; <5 years, <5 years old; PfDay0, Log *Plasmodium falciparum* density on day 0; TreatFail, Treatment failure outcome. Odds ratio were obtained from generalized linear and latent mixed model with binomial family using site as random effect; Covariates references were: CQ, AQ and SP for treatment, the age ≥5 years old for the age group, the 28-day cured parasite outcome after treatment for the treatment failure outcome, the baseline parasite density was log transformed and treated as continuous variable.

Multivariate analyses showed no risk of severe anaemia associated with artemisinin-derivative treatment arms (AS, AL, AS-AQ/AS-SP) compared to the non-artemisinin-contained treatments (CQ alone/AQ alone/SP alone/SP-AQ) from day 7 to day 28 (Figure 2).

Among a total of 956 eligible patients (with no severe anaemia before day 14 and haemoglobin data available on days 0, 7, 14, and 28), 38 patients (4%) experienced severe anaemia from day 14 to day 28. Multivariate analyses (Figure 2) showed no risk of severe anaemia associated with artemisinin-derivative treatment arms (AS, AL, AS-AQ/AS-SP) compared to the control non-artemisinin-contained treatments (CQ alone/AQ alone/SP alone/SP-AQ) from day 14 to day 28.

Among a total of 941 patients (with no severe anaemia before day 28 and haemoglobin data available on days 0, 7, 14, and 28), 23 patients (2.4%) experienced severe anemia on day 28. Multivariate analyses (Figure 2) showed no risk of severe anaemia associated with the artemisinin-derivative treatment arms (AS, AL, AS-AQ/AS-SP) compared to the control non-artemisinin-contained treatments (CQ alone/AQ alone/SP alone/SP-AQ) on day 28.

The risk of blood transfusion was assessed: a total of five cases of blood transfusion were documented, all in children ≤ three years old. Transfusion was done based both on low haemoglobin and clinical anaemia symptoms as judged by the physician. There were three in AS-AQ arm (one on day 2, one on day 3 and one on day 31), one in AS arm (on day 7) and one in SP arm (unknown day). Data of these patients were excluded from this analysis once they received the blood transfusion. The transfusion information was not available for one study (Maiga H *et al.* Dhfr-dhps quadruple mutant predicts sulfadoxine-pyrimethamine resistance in Mali, an emerging sulfadoxine-pyrimethamine resistance setting, unpublished observation) conducted in 2002–2004, which enrolled 948 patients in CQ arm.

Discussion

The study found no evidence of delayed anaemia risk associated with oral artemisinin derivatives. However, a moderate haemoglobin decrease or drop on day 7 was found. There were also risk factors associated with anaemia or haemoglobin decrease, such as treatment failure or high parasite density.

There are limited large-scale data addressing specifically haemoglobin changes or anaemia occurrence after oral artemisinin-derivative treatments. Recently, WHO recognized post-treatment haemolytic anaemia with artemisinin derivatives [10,11] and, therefore, called for the monitoring of haemoglobin as these data came from report cases from non-endemic malaria populations. This present study pooled individual data from 13 distinct studies involving 5,990 participants, of all age groups, from Mali, West Africa. The use of various artemisinin-containing treatments, including ACT and non-ACT offered the opportunity for comparison of haemoglobin changes or drop between different malaria treatments.

The baseline anaemia frequency was 36% and varied across the sites. It was lower than from a study [12], which reported an average frequency of 60% with pooled multi-country data, including part of data from Mali on artemisinin derivatives, which are also included in this study.

This study documented a transient and clinically moderate but significant decrease of haemoglobin on day 7 after treatment initiation in almost all malaria treatments. This finding was consistent with previous studies [24,25]. Mechanisms of anaemia or haemoglobin decrease following malaria episode are multiple, and are not fully understood. Various causes include haemolysis from malaria itself or from parasitized and non-parasitized red cells by the immune system or even from auto haemolysis mechanism [26,27]. Day 7 analysis, after adjusting for covariates (multivariate analysis) indicated that some ACT (AS or the combined AS-AQ/AS-SP/AS-PYR) compared to the non-ACT (CQ/AQ/SP) and covariates (age group < five years, high parasite density at baseline and treatment failure) were associated with higher haemoglobin decrease. Another study reported that younger age, high parasite density at baseline, and treatment failure were associated with the risk of post-treatment anaemia [12].

On day 14 and 28 there was significant increase in haemoglobin in most of treatment arms with maximum increase on day 28. The multivariate analyses indicated that ACT was not more associated with the risk of haemoglobin drop compared to non-ACT, while other covariates (such as treatment failure) were associated with the risk of haemoglobin drop. As reported in this study, haemoglobin increase or anaemia recovery, which is common by day 28, was also documented in several studies [12,24,25].

The frequency of severe anaemia, which was about 9% on day 0 and 10% on day 7, decreased gradually to 4% by day 28. Multivariate analyses indicated that there was a risk of severe anaemia associated with the AS arm from day 7 to day 28, compared to the non-ACT (CQ/AQ/SP/SP + AQ). But this was not evident from day 14 to 28 or on day 28. These observations could be explained by the relatively important decrease of haemoglobin shown in this study on day 7 particularly with AS arm (Table 3). Artemisinin derivatives have been shown to induce reticulocytopaenia in preclinical studies, potentially by suppressing the erythroblasts [27]. Although the reticulocytes have not been reported in this study, the relatively moderate and transient drop of haemoglobin, noted mainly on day 7, and the increase of haemoglobin from day 14 to day 28 suggest that oral artemisinin derivatives therapy may not have late clinically relevant deleterious effect on haemoglobin.

Following the previous study reporting anaemia without haemoglobin evolution assessment [12], this study had an

advantage of reporting haemoglobin evolution using a relatively large pooled malaria treatments database, including ACT, AS monotherapy and other non-artemisinin derivatives monotherapy, such as CQ, AQ and SP from various locations in Mali.

This study could not investigate the mechanisms involved in the haemoglobin evolutions observed. Indeed, the decrease on day 7 needs more study to improve the understanding of anaemia mechanisms, such as the reticulocytes evaluation. There is also a need for malaria treatment data from various countries, injectable AS treatment, vulnerable groups (pregnant women, HIV-infected subjects, patients with haemoglobinopathy, etc.).

Conclusions

No association was found between ACT and severe delayed anaemia. Oral artemisinin derivative treatments for uncomplicated *P. falciparum* malaria are associated with a transient and clinically moderate haemoglobin decrease by day 7 but not associated with a delayed severe anaemia.

Additional file

Additional file 1: Study treatment doses.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IS was the team leader for some of the clinical trials data involved in this study, conceived the study, prepared the data, carried out data analysis, wrote an initial draft of the manuscript, and worked on the final draft. RP contributed to the interpretation of the results and writing the manuscript. AD was the team leader for some of the clinical trials data, contributed to analyses and interpretation of the results, and to writing the manuscript. RG advised on data analysis and contributed to writing the manuscript. KK was the team leader for some of the clinical trials data, contributed to the interpretation of the results and writing the manuscript. OKD was the director of the clinical trials data, contributed to the study design, acquisition of community permission, interpretation of results, guided the data analysis and the writing of the manuscript. JG helped in the study conception, advised on data preparation, guided data analysis and interpretation of the results, and to writing the manuscript. All authors read and approved the final manuscript.

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Study Reference	Treatment information and doses
14	A single dose of 4 mg/kg Artesunate (Asunate Denk, Denk Pharma, Munich, Germany) was directly administered on the first day and a single dose of 2 mg/kg/day on each day thereafter. Doses were rounded up to the nearest quarter tablet. A full dose was re-administered in the case of rejection or vomiting in the first 30 minutes.
15	The Artemether-lumefantrine (AL) was Coartem (Novartis Pharma AG, Basel, Switzerland), given twice daily for 3 days to the following scheme: for patients < 15 kg, 1 tablet; 15–24 kg, 2 tablets; 25–34 kg, 3 tablets; ≥ 35 kg, 4 tablets.
16	Study drugs were given orally for 3 days (days 0, 1 and 2), dosed according to bodyweight. All doses were directly observed. Pyronaridine-artesunate was given once daily: ≥5 to <9 kg, one sachet; 9 to <17 kg, two sachets; 17 to <25 kg, three sachets (dose range 6.7/2.2 to 13.3/4.4 mg/kg/dose). Artemether-lumefantrine was given twice daily: ≥5 to <15 kg, one tablet; 15 to <25 kg, two tablets (dose range 1.3/8.0 to 4.0/24.0 mg/kg/dose); the second day-0 dose was 8 h after the first dose, the first day-1 dose was 24 h after the first day-0 dose, with all subsequent doses 12 h apart. Artemether-lumefantrine was given with food or milk as per local guidelines. Vomiting within

	30 minutes following the first drug dose resulted in re-dosing. Vomiting after repeat dosing or any subsequent dose resulted in study withdrawal and treatment with rescue medication (as per local guidelines).
17	Patients received either pyronaridine-artesunate once a day or artemether-lumefantrine twice a day, orally for three days (days 0, 1, and 2), plus respective placebo. Both treatments were given according to bodyweight (per dose for pyronaridine-artesunate: 20 kg to ≤25 kg one tablet, 26 kg to <45 kg two tablets, ≥45 kg to <65 kg three tablets, ≥65 kg to 90 kg four tablets, target 7·2/2·4 mg/kg to 13·8/4·6 mg/kg; per dose for artemether-lumefantrine: ≥20 kg to <25 kg two tablets, ≥25 kg to <35 kg three tablets, ≥35 kg to 90 kg four tablets). Food was not required for artemether-lumefantrine dosing to retain blinding. All doses were directly observed. Patients who vomited within 30 min could be re-dosed. If a patient vomited within 30 min of repeat dosing, he or she was withdrawn from the trial and given rescue medication (as per local clinical practice).
18	The dosages of Artesunate/Amodiaquine (Coarsucam™) were adapted according to the patient's body weight: • Body weight ≥10 and <18 kg: daily dosage of 50/135 mg. • Body weight ≥18 and <36 kg: daily dosage of 100/270 mg.

	• Body weight ≥36 kg: daily dosage of 200/540 mg. An interval of at least 8 hours must be maintained between the morning and the evening dose. Coarsucam™ is administered in 1 or 2 intakes per day, according to randomisation. The treatment duration is 3 days. The tablets are administered orally with a small amount of still drinking water. For the younger children, the tablets may be crushed and administered with still drinking water. **Artemether+lumefantrine (Coartem®):** The artemether/lumefantrine fixed-dose combination tablets contain 20/120 mg. The dosages of Coartem® were adapted according to the patient's body weight: • Body weight ≥10 and <15 kg: daily dosage of 40/240mg in 2 intakes • Body weight ≥15 and <25kg: daily dosage of 80/480mg in 2 intakes • Body weight ≥25 and <35 kg: daily dosage of 120/720mg in 2 intakes • Body weight ≥35 kg: daily dosage of 160/960mg in 2 intakes

	An interval of at least 8 hours must be maintained between the morning and the evening dose. The treatment duration is 3 days. The tablets are administered orally with still drinking water.
19	All study medicines were administrated orally by designated study personnel at the health center. The Artesunate+Amodiaquine (AS+AQ) was Arsucam® coblister (AS 50 mg/AQ 153 mg; Sanofi-Aventis, Paris, France), given once daily for 3 days to the following scheme: for patients < 10 kg, 1/2 tablet AS + 1/2 tablet AQ; 10–20 kg, 1 tablet AS+ 1 tablet AQ; 21–40 kg, 2 tablets AS+ 2 tablets AQ; > 40 kg, 4 tablets AS+ 4 tablets AQ. The Artesunate+Sulfadoxine-pyrimethamine (AS+SP) was Arsumax (AS 50 mg; Sanofi-Aventis, Paris, France) + sulfadoxine–pyrimethamine (S _ 500 mg/P _ 25 mg, Fansidar Roche, Burlington, NC), given once daily for 3 days to the following scheme: for patients ≤ 10 kg, 1/2 tablet AS+ 1/2 tablet SP; 11–20 kg, 1 tablet AS+ 1 tablet SP; 21–40 kg, 2 tablets AS+ 2 tablets SP; > 40 kg, 4 tablets AS+ 3 tablets SP. The SP is given only the first day, whereas AS is given over 3 days. The Artemether-lumefantrine (AL) was Coartem (Novartis Pharma AG, Basel, Switzerland), given twice daily for 3 days to the following

	scheme: for patients < 15 kg, 1 tablet; 15–24 kg, 2 tablets; 25–34 kg, 3 tablets; ≥ 35 kg, 4 tablets.
20	Treatment was administered according to body weight at the following doses: Amodiaquine: 10 mg/kg/day from day 0 to day 2; Artesunate: 4 mg/kg/day from day 0 to day 2; SP: 25 mg/kg of sulfadoxine and 1.25 mg/kg of pyrimethamine in single dose on day 0. All drugs were administered directly by the study team and the child was observed for 30 minutes. If vomiting occurred before 30 minutes, the dose was repeated; for vomiting after 30 minutes, a half-dose was administered. In the case of persistent vomiting, the child was referred to a health center for rescue treatment with intramuscular or intravenous quinine and withdrawn from the study.
21	Artemether-lumefantrine was packaged in fixed-dose combination tablets, each containing 20 mg of artemether and 120 mg of lumefantrine. They were administered according to body weight (5–14 kg, one tablet; 15–24 kg, two tablets; 25–34 kg, three tablets; ≥ 35 kg, four tablets) in six consecutive doses: one dose was administered at enrollment, one dose 8 hours later, and then two doses on the second day after initiation of treatment. The Artesunate+Mefloquine (AS + MEF) treatment was supplied as two separate tablets in a single blister pack: a tablet of mefloquine and a tablet of artesunate. One dose was

	administered daily for three days. Dosage was determined by weight, with different blister packs for different weight arms. For a weight ≥ 31 kg, each blister contained three 200-mg tablets of artesunate plus three 250-mg tablets of mefloquine. For a weight of 15–30 kg, each blister contained three 100-mg tablets of artesunate plus three 125-mg tablets of mefloquine. For a weight of 10–14 kg, artesunate has been given at a dose of 4 mg/kg and mefloquine at a dose of 5 mg/kg. For young children in both treatment arms, tablets were crushed and mixed with water. All drug doses were administered in the health center by a physician. A full dose was re-administered if a participant vomited the study drugs within 30 minutes of initial administration.
22	Artemether–lumefantrine was packaged in fixed-dose combination tablets each containing 20 mg of artemether and 120 mg of lumefantrine. They were administered according to body weight (5–14 kg, one tablet; 15–24 kg, two tablets; 25–34 kg, three tablets; ≥ 35 kg, four tablets) in four consecutive doses: one dose administered at enrollment, one dose eight hours later, and one dose on the first day and one dose on the second day after initiation of treatment. The Artesunate plus Sulfamethoxypyrazine-pyrimethamine treatment was supplied as two separate tablets in a single blister pack. The tablets were color-coded for ease of identification: pink for

	sulfamethoxypyrazine-pyrimethamine and white for artesunate. One dose was administered daily for three days. Dosage was determined by weight, with different blister packs for different weight arms. For a weight ≥ 40 kg, each blister contained a 200-mg tablet of artesunate plus a 500/25-mg tablet of sulfamethopyrazine-pyrimethamine. A tablet of each drug was administered each day. For a weight of 20–39 kg, each blister contained a 100-mg tablet of artesunate plus a 250/12.5-mg tablet of sulfametho-pyrazine-pyrimethamine. A tablet of each drug was administered each day. For weights of 13–19 kg, 8–12 kg, and 5–7 kg, each blister contained a 50-mg tablet of artesunate plus a 125/6.25-mg tablet of sulfamethopyrazine-pyrimethamine. One and a half tablets, one tablet, and half of a tablet, respectively, of each drug were administered daily. For young children in both treatment arms, tablets were crushed and mixed with water. All drug doses were administered in the health center by a physician. A full dose was re-administered if a participant vomited the study drugs within 30 minutes of initial administration.
23	Treatment#1: Atesunate/Amodiaquine (50 mg/153 mg; Arsucam® from Sanofi-Aventis, Paris, France), AS 4 mg/kg/day + AQ 10 mg/kg/day, 3 days of treatment. Treatment#2: Artesunate (50 mg; Arsumax® from Sanofi-Aventis) + Sulfadoxine/pyrimethamine (tablet of 500 mg/ 25 mg; from Roche). Artesunate 4 mg/kg/day for 3 days + Sulfadoxine/pyrimethamine 1

	tablet/20 kg, single dose administered on Day 0. Treatment#3: Artesunate (50 mg; Arsumax® from Sanofi-Aventis), monotherapy, 4 mg/kg on Day 0 followed with 2 mg/kg/day on Days 1–4.
24	Chloroquine was administered according to body weight at the following doses: 10 mg/kg/day on day 0 and day 1, 5 mg/kg/day on day 2.
25	Sulfadoxine-pyrimethamine was administered as a single dose of 25 mg/kg of sulfaadoxine and 1.25 mg/kg of pyrimethamine on day 0.
26	Chloroquine was administered at 25 mg/kg over three days (10 mg/kg on days 0 and 1, 5 mg/kg on day 2). Amodiaquine was administered at 25 mg/kg over three days (10 mg/kg on days 0 and 1, 5 mg/kg on day 2). Sulfadoxine-pyrimethamine was administered as a single dose of 25 mg/kg of sulfaadoxine and 1.25 mg/kg of pyrimethamine on day 0. All subjects were observed for 60 minutes to monitor for adverse reactions and to make sure that the medicine was not vomited. If vomiting occurred within 30 mn, the full dose was re-administered. If vomiting occurred after 30 mn a half dose was re-administered.

V. Discussions

Nos travaux ont couvert les différents aspects d'essais thérapeutiques de paludisme, à savoir l'efficacité thérapeutique, l'intérêt en santé publique d'un traitement antipaludique donné (effet de protection d'infection récurrente) quand le même traitement est donné à chaque épisode subséquent de paludisme et la sécurité ou tolérance (effet sur l'hémoglobine) des antipaludiques. Pour chaque problématique, des méthodes statistiques adaptées ont été mises en œuvre.

Pour l'analyse de données d'efficacité thérapeutique d'antipaludiques, la plupart des études de phase III, dont certaines réalisées par notre équipe, ont conduit à la prise de décision pour ces produits, notamment leur mise sur le marché au Mali et ailleurs [5-8, 28]. L'analyse des données de comptage des épisodes par bras de traitement selon le premier article [28] assume donc que les épisodes de paludisme chez la même patient sont indépendants. La limite de cette analyse est que les résultats peuvent être biaisés du fait que l'analyse n'avait pas tenu compte de la non indépendance d'épisodes de paludisme chez le même sujet [10].

En modifiant les critères de jugement, nous avons utilisé des outils statistiques appropriés pour l'analyse des épisodes récurrents ou répétés de paludisme pour répondre à la question d'intérêt en santé publique en montrant les médicaments qui étaient associés avec moins d'épisodes de paludisme pendant la période d'étude [28]. Nos travaux ont aussi permis d'identifier quelques modèles appropriés utilisables pour l'analyse des données récurrentes de paludisme [29]. Ce deuxième article [29] a analysé les mêmes données en utilisant quatre modèles statistiques après organisation attentive d'épisodes répétés de paludisme chez le même patient en précisant le délai d'apparition entre les épisodes tout en liant ces épisodes répétés au même patient durant toute l'étude. Parmi les quatre modèles [29], les estimations des coefficients de régression étaient similaires pour le modèle de fragilité partagée et le modèle d'AG-CP. Cependant, il est connu que le choix de distribution du modèle de fragilité

peut affecter les estimations des coefficients de régression et davantage de recherche est encore nécessaire dans ce domaine [10, 30].

En comparant les résultats des modèles du premier papier (régression binomiale négative) par rapport au ceux du deuxième papier (modèles de fragilité partagée d'AG-CP), nous obtenons de conclusion clinique similaire par rapport à l'effet de médicaments sur les événements récurrents même si cet effet était plus important avec les modèles de fragilité partagée et le modèle d'AG-CP.

Pour l'analyse des données de tolérance ou d'effets de médicaments sur l'hémoglobine, bien que nos travaux sont les premiers à regrouper autant de données individuelles d'essais cliniques à la recherche d'anémie retardée, post-thérapeutique d'accès palustre, de nombreuses publications sont faites sur l'analyse des données individuelles agrégées ou regroupées d'essais cliniques de paludisme pour l'évaluation de l'efficacité et de la tolérance [31-41]. Notre étude dans ce domaine avait une plus grande base de données évaluant la question d'anémie post-thérapeutique avec les dérivés d'artémisinine en zone d'endémie palustre.

L'outil statistique principal utilisé dans notre troisième papier [42] était le modèle GLLAMM qui a été déjà utilisé dans pour l'analyse de ce type de données dans l'évaluation de l'anémie [31], explorant les facteurs de risque. Notre étude n'a pas trouvé d'évidence d'anémie retardée associée avec la prise orale de dérivés d'artémisinine, ce qui est un résultat important en pratique clinique, ne modifiant pas les recommandations à propos des schémas thérapeutiques actuels. Nous avons cependant observé dans notre étude une baisse modérée et transitoire de taux d'hémoglobine au jour 7 comme l'ont trouvé aussi d'autres auteurs [24,41]. D'autres facteurs de risque associés avec l'anémie ou une baisse de taux d'hémoglobine tels que l'âge <5 ans, l'échec thérapeutique ou une densité parasitaire élevée au jour 0. Ce résultat était consistant avec les études antérieures [24,41].

VI. Perspectives

Suite à ce travail, les perspectives ou recommandations sont entre autres:

L'élaboration de guides de bonnes pratiques de références pour la méthodologie de préparation et d'analyse des données d'essais thérapeutiques de paludisme. Notamment, recommander l'analyse des événements récurrents avec l'un des 2 modèles appropriés (fragilité, AG-CP), dans le cadre des essais thérapeutiques d'épisodes récurrents de paludisme. Le réseau WANECAM pourrait assurer la diffusion de ces recommandations, et leur évolution en fonction des développements méthodologiques.

La création d'un entrepôt de données où seraient déposées toutes les données individuelles d'essais thérapeutiques de paludisme. Le réseau WANECAM (ROAMA : Réseau Ouest Africain de Développement des Médicaments Antipaludiques) pourrait en assurer la coordination.

Il s'agit d'un réseau de recherche et de développement des médicaments antipaludiques avec un financement de l'European and Developing Countries Clinical Trials Partnership (EDCTP). Ce Réseau regroupe quatre pays africains (Burkina Faso, Gambie, Guinée et Mali) et quatre pays Européens (Allemagne, Angleterre, France et Suède). Ce réseau transfrontalier est à même de contribuer effectivement au développement de nouveaux médicaments antipaludiques pour les besoins des populations.

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Annexe

Intitulés des doctorats AMU

Mentions et Spécialités des doctorats votés en CS le 16/10/2012

ED 62–SCIENCES DE LA VIE ET DE LA SANTE

- Biologie
    - Biochimie structurale
    - Génomique et Bioinformatique
    - Biologie du développement
    - Immunologie
    - Génétique
    - Microbiologie
    - Biologie végétale
- Neurosciences
- Pathologie humaine
    - Oncologie
    - Maladies infectieuses
    - Génétique humaine
    - Conseil en Génétique
    - Pathologie vasculaire et nutrition
    - Ethique
    - Recherche Clinique et Santé Publique

ED 67–SCIENCES JURIDIQUES ET POLITIQUES

- Droit privé
- Droit public
- Histoire du droit
- Droit
- Science politique

ED 184–MATHEMATIQUES ET INFORMATIQUE

- Mathématiques
- Informatique
- Automatique

ED 250–SCIENCES CHIMIQUES DE MARSEILLE

- Sciences chimiques

ED 251–SCIENCES DE L'ENVIRONNEMENT

- Anthropologie biologique