AQUAMAT; an open randomised comparison of artesunate versus quinine in the treatment of severe falciparum malaria in African children

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Abstract

Background

Severe malaria is a major cause of childhood death and a predominant reason for hospital admission in Sub-Saharan Africa. Quinine is still the established treatment of choice but recent evidence from a large multicentre trial in Asia suggests that artesunate is associated with a lower mortality.

Methods

An open label randomised comparison of parenteral artesunate and quinine in children with severe falciparum malaria was conducted in 11 centres in 9 African countries between October 2005 and July 2010.

Results

In total 5425 children were enrolled. The mortality in artesunate recipients was 8.5% (230/2712) compared with 11.0% (297/2713) in quinine recipients, an odds ratio stratified for study site of 0.75 (95%CI 0.63 to 0.90), and a relative reduction of 22.5% (95% CI 8.1 to 36.9%, p=0.002). There were no differences in the incidence of neurological sequelae, but both the development of coma (OR 0.68 ; 95% CI 0.49 to 0.94) and convulsions (OR 0.80 ; 95% CI 0.66 to 0.97) were significantly less frequent in artesunate recipients (both p=0.02). Hypoglycaemia after starting antimalarial treatment was less frequent in artesunate recipients 48/2712 (1.8%) compared with artesunate 75/2713 (2.76%); OR 0.63 (95% CI 0.43 to 0.91, p = 0.013). Artesunate was well tolerated and there were no serious drug related adverse effects.

Conclusions

Artesunate substantially reduces mortality in African children with severe malaria. These data, together with a meta-analysis of all trials comparing artesunate and quinine, strongly suggest that parenteral artesunate should replace quinine everywhere as the treatment of choice for severe falciparum malaria.

Clinical trial registration number ISRCTN50258054.

Introduction

Falciparum malaria is a major killer of African children and one of the main causes of paediatric hospital admission across sub-Saharan Africa. Many deaths occur in or near home, but for those children who are admitted to hospital with severe malaria and receive parenteral antimalarial treatment, approximately one in six will die[1,2]. Quinine was the mainstay of severe malaria treatment for over 300 years from the time of its introduction to European medicine in the 1630s until the deployment of parenteral chloroquine in the 1950s. Resistance to chloroquine emerged in SouthEast Asia but then spread to Africa at the end of the 1970s, and quinine then resumed its primary role in the treatment of severe malaria. Parenteral quinine is given either by intramuscular injection or slow intravenous infusion[1,2]. It has a narrow therapeutic ratio[3]. Intravenous quinine administration requires a constant rate infusion with dosing three times daily. Intramuscular administration is painful, and may cause sterile abscesses and predispose to lethal tetanus[4]. Although blindness and deafness may follow self poisoning, they are rare in severe malaria, but quinine induced hyperinsulinaemic hypoglycaemia is a particular problem in patient management especially in pregnant women[1,2,5].

The primacy of quinine in the treatment of severe malaria has been challenged in recent years by introduction of the artemisinin derivatives. The initial comparative clinical trials were conducted with intramuscular artemether, a lipophilic derivative of dihydroartemisinin (DHA). These showed that artemether was safer and easier to use than quinine, but in an individual patient data meta-analysis of 1919 randomised patients, overall survival was not significantly different between quinine and artemether recipients[7]. In a prospectively defined sub-group analysis artemether did reduce mortality significantly in SE Asian adults, but it did not do so in African children. Artemether is an oil-based formulation which releases the drug slowly and erratically from the injection site [8-11] whereas the water-soluble hemisuccinate artesunate can be given intravenously and is absorbed reliably and rapidly following intramuscular injection with peak concentrations occurring within one hour [11-13]. A large multicentre randomised trial (SEAQUAMAT), which compared artesunate with quinine in Asian patients (most of whom were adults) with severe malaria, was stopped by its data safety monitoring committee after enrolment of 1461 patients because of a substantial survival benefit in favour of artesunate[14]. A meta-analysis of the SEAQUAMAT study and smaller, earlier randomised trials showed that artesunate reduced the mortality

of severe malaria in Asian patients from 22% to 15%, a relative reduction in mortality of 35%. The treatment was also highly cost-effective[15]. In 2006, the World Health Organisation changed its guidelines to recommend artesunate for severe malaria for adults[16].

The treatment effect in the SEAQUAMAT trial was similar in adults and the 202 children enrolled, but perceived differences in the natural history and drug susceptibility of severe falciparum malaria in African children compared with Asian patients, left uncertainty over the optimum treatment for this most important patient group. Expert opinion considered parenteral quinine to be a satisfactory treatment, and research priorities focussed more on improving other aspects of the care of the sick child. As a result quinine remains by far the most widely used treatment of severe malaria in Africa. We report here a very large multicentre randomised trial which compared artesunate and quinine treatment of severe malaria in African children.

Methods

This was a multicentre open label comparison of parenteral artesunate and parenteral quinine in children admitted to hospital with severe malaria. Most children were managed on general paediatric wards. The eleven participating centres in nine countries across Africa were: Hospital Central da Beira, Beira, Mozambique; Royal Victoria Teaching Hospital, Banjul, The Gambia; Komfo Anokye Hospital, Kumasi, Ghana; Kilifi District General Hospital, Kilifi, Kenya; Magunga District Hospital, Korogwe, Tanzania; Teule District Hospital, Muheza, Tanzania; University of Ilorin Teaching Hospital, Ilorin, Nigeria; Mbarara Teaching Hospital, Mbarara, Uganda; Kingasani Health Centre, Kinshasa, DRC; Rwamagana Hospital, Rwamagana, Rwanda and Nyanza Hospital, Nyanza, Rwanda. The study was coordinated by the Mahidol-Oxford Research Unit in Bangkok which provided logistic support and data management. Children less than 15 years were included in the study if they had a positive rapid diagnostic test (RDT) for *Pf*LDH (Optimal™) and, in the admitting physician's clinical opinion, they had severe malaria, and they or their attendant relative or guardian gave fully informed written consent. Patients were not included if there was a convincing history of full treatment with quinine or an artemisinin derivative for more than 24 hours before admission. For quinine this was defined as 40 mg salt/kg on the first day and 30mg/kg on any subsequent day. Age criteria varied slightly among sites at the request of the respective

ethical review boards (ERBs) (see annexe). In Mozambique adults were also included in the study but these were analysed separately and will be reported elsewhere.

Sample size.

The aim was to include at least 5306 children with severe malaria, in order to show a 25% reduction in mortality from an estimated 8% to 6% with a power of 80% and a confidence of 95%. This estimate was based on approximately half the enrolled patients fulfilling subsequently modified WHO criteria for severe malaria[17], and these severely ill children having a 16% mortality. Patient data and outcomes were provided to an independent data and safety monitoring committee who conducted annual interim analyses.

Antimalarial treatment and randomisation.

Patients were randomized to treatment with either intravenous or intramuscular artesunate or quinine. Each centre had a policy of using one route of administration.

Artesunate (Guilin Pharmaceutical Factory, Guangxi, People's Republic of China) was given in a dose of 2.4 mg/kg body weight on admission, then at 12 hours, 24 hours and thereafter once daily until oral medication could be taken reliably. Each 60 mg vial of artesunic acid was dissolved initially in 1 ml 5% sodium bicarbonate and then diluted with 5% dextrose before injecting the weight adjusted dose as a bolus into an indwelling intravenous cannula, or was given by deep intramuscular injection to the anterior thigh. When the patient was able to take tablets, oral artemether-lumefantrine (Novartis, Switzerland) in a full standard dose (1.5/9mg/kg twice daily for three days with milk or fat) was given to complete the treatment.

Quinine dihydrochloride (Indus Pharma (Pvt.) Ltd. Karachi, Pakistan) was given in a 20 mg salt/kg loading dose infused over 4 hours (in 5 to 10 mL/kg 5% dextrose water), followed by a 10 mg salt/kg infusion over 2-8 hours three times a day until starting oral therapy. The doses for intramuscular treatment were the same as for intravenous treatment. The drug was diluted in normal saline, to provide a concentration of 60 to 100 mg per millilitre, and injected in the anterior thigh. The loading dose was given as a split dose into each thigh. The follow-up treatment with artemether-lumefantrine was the same in both groups.

Randomisation was done by study site in blocks of twenty. The study numbers were kept inside an opaque sealed paper envelope. Although the trial was open label at each site, none of the investigators or trialists, with the exception of the trial statistician, had access to the summaries of treatment allocations. When notes or case record forms were reviewed the parenteral antimalarial drug allocations were removed to preserve blinding. All case reviews and laboratory assessments were conducted blinded to treatment allocation. The treatment allocations were not revealed until the database was locked at the end of the trial.

Procedures.

Patients with suspected severe malaria were examined, admitted, and the diagnosis of malaria was confirmed by RDT. After full informed written consent was obtained, the next envelope was opened, and the enclosed unique study number was allocated. Then the corresponding numbered sealed box was opened. This contained the study drug, case record form, and all disposables needed for drug administration and blood sampling. A 1mL blood sample was taken for immediate haematocrit and biochemical analysis using the EC8+ card for a hand held blood analyser (i-STAT®, Abbott Laboratories, Il, USA). This produced an immediate printed paper report with time of day which was kept with the case record form. Haemoglobin was estimated from the i-STAT result or when not available calculated from the local hospital haematocrit (n=146), using an MCV of 80 fL, and an MCH of 1.7 fmol/cell. Peripheral thick and thin blood smears were made for later quantitative malaria parasite counting, at the reference laboratory in Bangkok[14,18,19] . Parasites were quantified using the readings from the thin film, or if not available calculated from the thick film (40 x count/200WBC). In 109 cases no quantative reading was available from the reference laboratory, and the parasitaemia reported by the study site was used. Treatment was started immediately. All other aspects of supportive treatment, based on WHO guidelines, were unaffected by the trial[1,2,16]. Children were discharged from hospital at the discretion of the physician, and a discharge assessment was completed by the study team. Children who did not have full neurological recovery by discharge were followed up as an outpatient at regular intervals for 12 months or until full recovery occurred. Full neurological assessments were completed at each visit. Training in neurological assessment was provided to all sites by a specialist paediatric neurologist to ensure uniformity.

Ethical review

The trial protocol was reviewed and approved by each site's appropriate ethical review board (ERBs), and also by the Oxford Tropical Research Ethics committee (OXTREC). Permission to investigate HIV status was obtained only from some ERBs.

Monitoring and quality assurance

Trial sites were monitored regularly by Family Health International. Investigators provided reports every two weeks to the coordinating centre in Bangkok and met each year to review study progress. Drug content and quality were checked in ampoules randomly taken from the purchase lots. The DSMC also made periodic checks of clinical and laboratory parameters and other aspects of management to determine if randomisation was adequate.

Analysis

The analysis was conducted according to a pre-specified analytical plan. The primary outcome measure was in-hospital mortality compared between treatments on an "intention to treat" basis. Secondary outcomes measures were the incidence of severe complications or persistent neurological sequelae (assessed at 28 days, range 3 to 8 weeks) and a combined outcome measure of death and severe persistent neurological sequelae. Initially neurological sequelae were assessed only at discharge from hospital, but it soon became apparent that this led to significant overestimation of neurological deficit, particularly in young children. The protocol was therefore changed so that children who had not yet fully recovered at discharge were to be assessed 28 days after enrolment, and active follow up was instituted. Outcome measures were also assessed on a "per-protocol" basis, excluding patients not fulfilling the entry criteria, patients with a negative or missing peripheral blood slide for *P. falciparum* on admission, patients dying before the study drug was administered, and those missing a study drug dose on the first day of treatment (Figure 1). The incidence of complications including persistent neurological sequelae, hypoglycaemia (glucose <3 mmol/L), haemoglobinuria, and any other severe and unexpected adverse event were also compared. Subgroup analyses included stratification into two predefined groups: those fulfilling WHO criteria for severe malaria modified by Hien *et al.* [1,2,] , (see table 1) and those who were entered into the study on clinical grounds, but were later found not to fulfil these

criteria. Other predefined analyses included the comparison between slide positive and negative patients, presence or absence of pretreatment with antimalarials, comparing study sites with mortality above or below the average of the study, intramuscular versus intravenous treated patient groups, presence or absence of hyperparasitaemia, coma, shock, severe anaemia (<5 g/dl), respiratory distress, and severe acidosis (BE<8 mmol/L) on admission. In sites where testing was approved the HIV status of participants was assessed after obtaining a separate informed consent. In a subgroup analysis the outcomes in HIV-1 infected malaria patients were compared acoording to treatment allocation.

Assessment of outcomes

To provide independent expert assessment and classification of outcomes before study unblinding two committees were formed. A neurological outcome committee, comprising two experienced paediatricians (both with neurological expertise and experience working in Africa) and one clinical malariologist, graded neurological sequelae. Sequelae were assessed in four domains: motor function, visual function, hearing and speech and also whether the child had developed epilepsy. Sequelae persisting at follow up were graded as mild, moderate or severe in each domain according to the functional impairment associated with the description (Annexe). The overall grade was combined and the presence of sequelae in more than one functional domain resulted in a step up in grading. Relation of sequelae to severe malaria was assessed depending on the presence of comorbidity. A separate end-point review committee, comprising one paediatrician with malaria experience and one clinical malariologist, identified cases where a pathology other than malaria or its acute complications was the main cause of death and thus not preventable by a better antimalarial drug.

Statistical methods

For binary outcomes, the odds ratios for death between treatment groups, stratified by study site, were estimated using Mantel-Haenzel method. Heterogeneity between sites was examined with Breslow-Day test. In Ruanda the two small sites run by the same investigators were pooled. For “time to event” outcomes, hazard ratios were estimated by a Cox proportional hazard model, stratified by site, and were compared with log rank test. Statistical analyses were performed using Stata®v11.1 (Statacore, College Station, TX, USA).

RESULTS

The trial took place between October 2005 and ended in July 2010 when it was confirmed that the recruitment target had been reached. The trial recruitment progress by site is shown in the annexe. During the trial, additional study sites were included to increase recruitment. The study profile is shown in Figure 1.

Baseline characteristics

There were no major imbalances in baseline characteristics between the two treatment groups (Table 2), indicating that randomisation was successful.

Mortality

In total 5425 patients were recruited (Figure 1) of whom 527 (9.7%) died. The intention to treat mortality was 8.5% (230/2712) in the artesunate recipients compared with 10.9% (297/2713) in quinine recipients, a relative risk (95% CI) of 0.77 (0.66 to 0.91); p=0.002. This represents a reduction in mortality of 22.5% (95% CI 8.1 to 36.9%). The odds ratio (95% CI) was 0.75 (0.63 to 0.90) in favour of artesunate (p=0.002). There was no heterogeneity between the study sites (p=0.99). Eight patients left hospital against advice and could not be followed-up further. These patients were censored in the survival analysis at the time of discharge. The Kaplan Meier survival curve (Figure 2) for overall mortality during hospitalisation by antimalarial treatment gives the same result as the Mantel-Haenzel analysis (log rank test stratified by study site: p=0.002). Cox regression analysis of the pre-defined subgroups showed no evidence of any differences in odds ratios between subgroups (Figure 3). The per protocol analysis excluded the 28 patients who died rapidly before receiving the prescribed antimalarial treatment, patients with incomplete initial antimalarial treatment, and those with a negative or missing blood slide for *P. falciparum* (numbers in Figure 1), but these omissions also did not materially affect the result (Table 3). The end point review committee identified 16 children (9 quinine; 7 artesunate) in whom death was unlikely to be related to severe malaria. Omitting these cases from the analysis also had no effect on the magnitude of the survival benefit with artesunate (Table 3). The difference in case fatality rate in favour of artesunate was larger in patients who died after 24 hours in hospital; odds ratio (95%CI) of 0.64 (0.47 to 0.87), than in children who died within the first 24 hours after admission; odds ratio 0.83 (0.66 to 1.03). HIV serology was

assessed routinely in Beira, Mozambique, Muheza, Tanzania and Kilifi in Kenya. Of 2095 patients tested, 69 (3%) were positive. The median (range) percentage of CD4+ lymphocytes was 21% in both groups with a range of 1.7 to 55%. Mortality in these patients was high; 35% with quinine, and 25% with artesunate treatment (Table 3).

Neurological outcomes and sequelae

In total 497 children had convulsions which either developed or persisted for more than six hours after admission, and 157 patients developed coma or had a deterioration of their coma score after the start of antimalarial treatment (Table 4). Both coma and convulsions developed more frequently in quinine recipients (both p=0.02). Among the 4898 survivors, 170 children had not yet made a full neurological recovery at the time of hospital discharge (Figure 4). 129 (76%) of these children were followed up between 3 and 8 weeks after enrolment. At the first follow up assessment 68 (53%) had recovered fully, 18 (14%) were considered mildly or moderately impaired, and 43 (33%) were considered to have severe neurological deficits; 20 had received quinine and 23 artesunate (p= 0.75). The overall incidence of any persistent neurological sequelae at 28 days following cerebral malaria was 47 out of 1443 (3.3%) surviving patients and of severe neurological sequelae 34/1443 (2.4%) and there was no difference between the treatment groups. Of the 14 patients with neurological sequelae who did not present initially with coma, 7 had multiple convulsions and 7 had severe prostration on admission.

Adverse events and other sequelae

There were no severe adverse effects which could be attributed directly to drug toxicity. One patient treated with artesunate developed a mild urticarial rash, but no severe type 1 hypersensitivity reactions to artesunate were observed. Another patient treated with artesunate developed peripheral gangrene of toes and fingers –this was attributed to the disease and not the drug. One patient treated with quinine developed a severe stridor with severe respiratory insufficiency after administration of ampicillin, and died. This was attributed to ampicillin and not quinine. Other complications during admission are listed in Table 4. Hypoglycaemia occurring after starting antimalarial treatment was less frequent in artesunate recipients 48/2712 (1.8%) than in quinine recipients 75/2713 (2.8%); OR 0.63 (95% CI 0.43 to 0.91), p = 0.013. Blackwater fever was a rare event in both groups.

Additional treatments

Blood transfusions were given to 1495/2713 (55%) patients treated with quinine and 1487/2712 (55%) with artesunate. A fluid bolus at the start of treatment was given in 596/2713 (22%) patients receiving quinine and 589/2712 (22%) with artesunate. In total 3259 (60%) children received antibiotics, equally distributed between the two treatment groups.

Time to recovery

In the surviving patients who presented initially in coma, recovery, defined as the time since randomisation until the child was able to localize a painful stimulus (median 12 hours in both treatment groups) or able to speak (in children old enough to do so; median 18 hours quinine, 20 hours artesunate) was usually within the first day, but was slightly longer in patients treated with artesunate than with quinine (Table 5). The times to eat or to sit unsupported did not differ between treatment groups.

Quality assessments of drugs used

The content of artesunate in all ampoules tested was found to be within the pharmaceutical content limits of 10% of the stated active ingredient. All tested vials of quinine contained between 105-112% of the stated content (see annexe)

Meta-analysis of trials

A meta-analysis of all trials which have compared survival in patients with severe malaria treated with parenteral artesunate versus quinine is shown in Figure 5. The overall odds ratio (95%CI) is 0.69 (0.57 to 0.84, p=0.000001). There is no significant heterogeneity between the results generated in Africa and Asia (p=0.10).

DISCUSSION

This multi-centre trial is considerably larger than any other treatment trial in patients hospitalised with severe malaria. It shows that artesunate reduces the overall mortality of African children diagnosed with severe malaria by 22.5% (95% CI 8.1 to 36.9%). There was little heterogeneity between treatment centres in the benefit associated with artesunate,

indicating that the findings are robust. Before this trial there were concerns that artesunate might not be superior to quinine in African children because of perceived differences in pathology, prevalence of more quinine susceptible malaria parasites, and that in the earlier SEAQUAMAT trial conducted in Asia, the survival curves did not clearly separate until 48 hours after starting treatment, whereas the majority of deaths in African children occurred earlier. In AQUAMAT the survival curves diverged earlier. This large benefit in mortality is consistent with previous studies. Over 7000 severely ill patients have now been included in randomised comparisons of parenteral artesunate and quinine. The meta-analysis of all trials (Figure 4) shows a consistent, highly significant, substantial benefit, so there can be considerable confidence in the superiority of artesunate over quinine in the treatment of severe malaria.

Artesunate prevented death from severe malaria, but this was not at the expense of an increase in neurological sequelae. Indeed the overall incidence of confirmed persistent severe sequelae following severe malaria was low (less than 1% of cases overall, 2.4% following cerebral malaria). Initially the assessment of neurological deficit was made at discharge from hospital, but it soon became apparent that many children, particularly in the younger age group, had not made a full neurological recovery at discharge. Subsequently assessments were made four weeks later by which time approximately half the apparent deficits had resolved fully.

Compared with parenteral quinine, artesunate reduced the mortality of severe malaria in African children by 22.5%, and in Asian patients by 35%[14]. Compared with placebo pre-referral treatment with rectal artesunate reduced the mortality of severe malaria by 25% in children[21]. These studies comprise together over 80% of all patients ever enrolled in randomised controlled trials of severe malaria, and they provide definitive evidence that artesunate is the best available treatment for severe malaria, and that it is safe. This life-saving benefit over quinine in severe malaria must derive from greater intrinsic parasiticidal activity. The principal pharmacodynamic advantage of artesunate over quinine is the much broader stage-specificity of action of the artemisinin compounds[21]. These drugs kill circulating ring-stage parasites before they can mature and cause sequestration of the infected erythrocytes in the venules and capillaries of vital organs and, as a result, potentially lethal microvascular obstruction[22-26]. The large and consistent

reduction in mortality associated with artesunate supports the central quantitative role of parasitized erythrocyte sequestration in the pathology of malaria.

The benefits of parenteral artesunate over quinine were greater than the benefits of intramuscular artemether reported in earlier trials. Compared with quinine, artemether reduced mortality significantly in Asian studies (studies which recruited predominantly adults) but had no significant benefit in African children[7]. In a recently reported double blind trial comparing artesunate and artemether in 370 Vietnamese adults with severe falciparum malaria there were 13 deaths in the artesunate group (7%) and 24 in the artemether group (13%) [27]. Taken together these data suggest that artesunate provides approximately twice the live-saving benefit provided by artemether. The two compounds have similar pharmacodynamic properties in-vitro so the most likely explanation for their different efficacies is the marked difference in the pharmacokinetic properties of the two artemisinin compounds[11]. Artemether is an oil-based formulation absorbed slowly and erratically following intramuscular injection whereas both artesunate and quinine are water soluble and are absorbed rapidly and reliably following intramuscular injection[11-13], and both can be given intravenously. Thus the pharmacodynamic advantage of artemether may have been offset by its poor absorption kinetics. In summary, in the treatment of severe falciparum malaria, artesunate is superior to artemether, which in turn is superior to quinine.

The benefits of artesunate (and artemether) over quinine were greater in patients from South-East Asia than in African children[14]. There are several possible explanations for this difference. African children usually have some degree of background immunity which assists the therapeutic response and accelerates recovery, and this may reduce the therapeutic advantage of artesunate. The greater quinine susceptibility of *Plasmodium falciparum* on the African continent is often proposed as a contributory factor to differences in therapeutic responses, but differences are not large, and are unlikely to have a major effect in-vivo. Incorrect diagnosis leading to misclassification is likely to be a major contributory factor. Severe malaria is overdiagnosed in African children, and sepsis is underdiagnosed[28]. Febrile sick children with positive malaria blood smears are usually diagnosed initially as severe malaria, but the specificity of a positive blood smear is poor in settings where a high proportion of all children are parasitaemic. Septicaemia and pneumonia are particularly difficult to differentiate clinically from severe malaria. Sepsis and

severe malaria also commonly coexist in African children. Prompt and appropriate treatment of sepsis should further reduce the mortality of severely ill parasitaemic children.

Artesunate should now become the treatment of choice for severe malaria for children and adults everywhere in the world. Artesunate is simple to administer, safe, and it reduces mortality significantly compared with quinine. No serious adverse effects were identified in this large study or in previous studies. By contrast quinine is locally toxic (because of its acidity) following intramuscular injection, cannot be given by bolus intravenous injection, requires three times daily administration, is associated with potentially serious hypoglycaemia, and is much less effective in the treatment of severe malaria. In this trial 22 children died before receiving quinine compared with only six who died before receiving artesunate (p=0.002). This probably reflects the difficulties in administering parenteral quinine safely and in a timely matter. Any delay in treating severe infection will increase mortality. The ease and safety of parenteral artesunate are important practical advantages. Artesunate is more expensive to buy but quinine is more expensive to administer. An analysis of the relative cost-effectiveness of artesunate and quinine is currently underway. A major factor limiting the deployment of artesunate has been the availability of a product satisfying international good manufacturing standards. The most widely used product, which was evaluated in this study, does not yet have this certification. This has prevented deployment in some countries[29-34]. This hurdle must be overcome speedily so that parenteral artesunate can be deployed in malaria endemic areas and lives saved.

Malaria causes an estimated 800,000 deaths each year in African children[20]. Severe malaria is often the most common admission diagnosis in febrile children, so a change in treatment policy from quinine to artesunate has the potential to save thousands of childrens' lives each year. If four million of the African children with severe malaria each year were to be treated with parenteral artesunate instead of quinine, and the benefits were similar to those observed in this trial, then approximately 100,000 lives could be saved annually.

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The coordinating committee designed the study

All investigators in the trial sites conducted the trial with support from the team in Bangkok.

All investigators and coordinating committee reviewed and discussed the trial results.

The writing committee conducted the data analysis and prepared the report.

Conflict of interest statements

NJ White is co-chairman and Olugbenga Mokulu is a member of the WHO antimalarial treatment guidelines committee. None of the other authors have any conflicts of interest. NJW had full

access to all the data in the study and had final responsibility for the decision to submit for publication.

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Tables

Table 1.

Modified criteria for severe falciparum malaria:

At least one of:

1. Plasma Base Excess <-3.3 mmol/L
2. Glasgow coma scale <11/15 or Blantyre Coma Scale <3/5 in preverbal children.
2 Haemoglobin < 5 mg/dL and parasitaemia > 100,000 parasites/uL
3. Blood urea nitrogen > 60 mg/dl.
5. Compensated shock (capillary refill >=3 sec or temperature gradient)
6. Decompensated shock. Systolic blood pressure < 70 mm Hg and cool peripheries.
7. Asexual parasitaemia > 10%.
8. Visible jaundice and more than 100,000 parasites/uL
9. Plasma glucose < 3 mmol/L.
10. Respiratory distress, defined by costal indrawing or respiratory insufficiency.

Table 2.

Baseline characteristics in the two treatment group. Values are mean (SD), unless otherwise indicated.

Variable	Quinine (N=2713)	Artesunate (N=2712)
Gender; female (n, %)	1295 (48%)	1315 (48%)
Age (years, median, IQR)	2.9 (1.7 to 4.3)	2.8 (1.6 to 4.2)
Fever before enrollment (days, meduan, IQR)	3 (2 to 4)	3 (2 to 4)
Coma before enrollment (h, median, IQR)	5.0 (2.5 to 10)	5.0 (2.0 to 9.5)
Pre-treatment with antimalarials (n, %)		
None	1270 (47%)	1281 (47%)
Ineffective	268 (10%)	290 (11%)
Moderately	103 (4%)	97 (4%)
Effective	959 (35%)	938 (35%)
Complications on admission		
*Coma	997 (37%)	943 (35%)
Convulsions	879 (32%)	811 (30%)
Jaundice	59 (2%)	55 (2%)
Severe anaemia (<5g/dL)	693 (29%)	738 (30%)
Compensated shock	251 (9%)	233 (9%)
Decompensated shock	88 (3%)	90 (3%)
Severe acidosis (BE <-8 mmol/L)	975 (43%)	1009 (44%)
Hypoglycaemia (<3 mmol/L)	278 (10%)	277 (10%)
**Respiratory distress	428 (16%)	439 (16%)
***Severe prostration	1668 (61%)	1683 (62%)
Blackwater fever	116 (4%)	121 (4%)
Hyperparasitaemia (>10%)	573 (24%)	584 (25%)
Clinical examination		
Weight (kg)	12.6 (4.6)	12.4 (4.8)
Temperature (°C)	38.0 (1.1)	38.0 (1.1)
Blood pressure (mmHg)		
Systolic	95 (16)	95 (16)
Diastolic	56 (14)	56 (14)
Coma depth (total N, median, range)		
Blantyre Coma Score	*N=1704*, 4 (0 to 5)	*N=1713*, 4 (0 to 5)
Glasgow Coma Score	*N=1005*, 11 (3 to 15)	*N=999*, 11 (3 to 15)

Table 2 (continued)

Variable	Quinine (N=2713)	Artesunate (N=2712)
Comorbidity (n, %)		
Chronic disease	62 (2%)	49 (2%)
Immune compromised	49 (2%)	45 (2%)
Severe malnutrition	43 (2%)	54 (2%)
Suspected pneumonia	223 (8%)	224 (8%)
number confirmed by x-ray	29 (13%)	29 (13%)
Clinical sepsis	355 (13%)	302 (11%)
number confirmed by culture	33 (9%)	32 (11%)
Suspected meningitis	166 (6%)	169 (6%)
Confirmed meningitis	3 (2%)	6 (4%)
Malnutrition	28 (1%)	34 (1%)
Other significant comorbidities	71 (3%)	80 (3%)
Laboratory assessments		
Potassium (mmol/L)	4.1 (0.9)	4.1 (0.9)
Chloride (mmol/L)	105 (10)	105 (10)
Blood Urea Nitrogen (mmol/L)	6.1 (4.9)	6.1 (4.6)
Haemoglobin (g/dl)	7.0 (3.1)	6.8 (2.9)
pH	7.36 (0.14)	7.36 (0.14)
PaCO2 (mmHg)	28.2 (10.1)	27.9 (9.1)
HCO3 (mmol/L)	16.6 (5.7)	16.6 (5.6)
Base excess (mmol/L)	-9 (7)	-9 (7)
Anion gap (mmol/L)	17 (5)	17 (5)

*Depth of coma was assessed either as Blantyre Coma Score (for younger children, n=3417) or Glasgow Coma Scale (n=2004).

**Respiratory distress was defined as costal indrawing or respiratory insufficiency

*** severe prostration was defined as inability to breastfeed under 6 months old or inability to sit in older children.

Table 3.

Main outcomes, death attributable to malaria and case fatality in HIV positive children according to treatment group.

Variable	Quinine (n/total, %)	Artesunate (n/total, %)	OR (95% CI)	p-value
Mortality, "ITT" analysis	297/2713 (10.9%)	230/2712 (8.5%)	0.75 (0.63 to 0.90)	0.002
Mortality, "per protocol" analysis	260/2552 (10.2%)	208/2563 (8.1%)	0.78 (0.64 to 0.94)	0.009
Death or Sequelae at 28 days	297/2695 (11.0%)	230/2689 (8.6%)	0.78 (0.65 to 0.93)	0.006
*Malaria attributable mortality	288/2704 (10.7%)	223/2705 (8.2%)	0.75 (0.63 to 0.91)	0.002
Strictly defined severe malaria	291/2338 (12.4%)	226/2280 (9.9%)	0.77 (0.64 to 0.93)	0.005
**Case fatality in HIV positive children	19/61 (31%)	16/64 (25%)	0.74 (0.33 to 1.62)	0.446

ITT = intention to treat.

* The likelihood that malaria contributed to or directly caused the death was evaluated by an independent endpoint review committee blinded to the treatment allocation.
**HIV status was assessed only in Beira, Muheza, and Kilifi. (N=2095).

Table 4.

Complications developing after start of treatment, according to treatment group. Development of coma, anaemia and black water fever was only assessed in patients without this condition on admission.

Variable	Quinine (n/total, %)	Artesunate (n/total, %)	OR (95% CI)	p-value
Development of coma	92/1716 (5.4%)	65/1769 (3.7%)	0.68 (0.49 to 0.94)	0.020
Deterioration of coma score	155/2713 (5.7%)	130/2712 (4.8%)	0.83 (0.65 to 1.06)	0.127
Convulsions developing or persisting > 6h after admission	273/2713 (10.1%)	224/2712 (8.3%)	0.80 (0.66 to 0.97)	0.019
Hypoglycaemia	75/2713 (2.8%)	48/2712 (1.8%)	0.63 (0.43 to 0.91)	0.013
Severe anaemia (<5 g/dL) after admission	102/1631 (6.3%)	77/1581 (4.9%)	0.76 (0.56 to 1.04)	0.082
Black water fever	18/2597 (0.7%)	30/2591 (1.2%)	1.69 (0.94 to 3.05)	0.076

Table 5.

Time to events in surviving patients according to treatment group.

Variable	Quinine (median, IQR)	N	Artesunate (median, IQR)	N	HR (95% CI)	p-value
Time to discharge (days)	3.0 (2.0 to 5.0)	2412	3.0 (2.0 to 5.0)	2478	1.04 (0.99 to 1.11)	0.136
Time to eat (hours)	12 (2 to 24)	2269	9 (0 to 24)	2358	0.99 (0.93 to 1.06)	0.771
Time to sit unsupported (hours)	22 (6 to 44)	2312	18 (6 to 42)	2373	1.02 (0.95 to 1.08)	0.626
*Time to localize pain (hours)	12 (6 to 24)	726	12 (6 to 24)	698	0.87 (0.78 to 0.98)	0.021
*Time to speak (hours)	18 (11 to 36)	695	20 (8 to 42)	664	0.88 (0.79 to 0.99)	0.027

Time to localize pain and time to speak was assessed only for surviving patients with coma on admission (BCS<3 or GCS<11).

Legends to figures

Figure 1

AQUAMAT trial profile

Figure 2

Neurological sequelae at discharge and after 28 days (range 3 weeks to 8 weeks) in children with severe falciparum malaria enrolled in the AQUAMAT trial.

Figure 3

Kaplan Meier curves comparing survival in African children with severe falciparum malaria treated with either parenteral artesunate or quinine. The numbers in brackets are the case fatality rates during the indicated time period.

Figure 4

Meta-analysis of all randomised controlled trials which have compared parenteral artesunate and parenteral quinine in severe malaria.

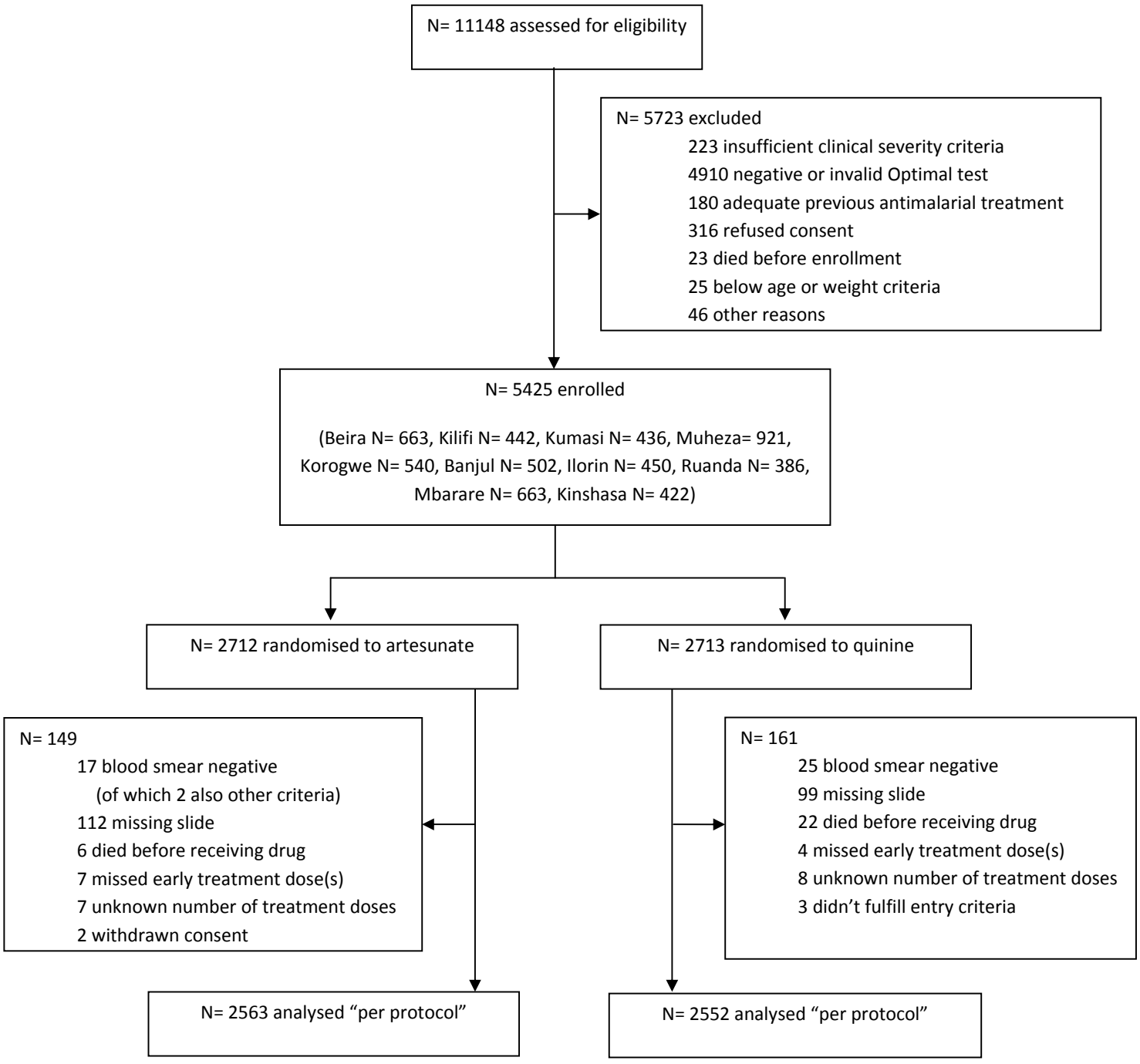
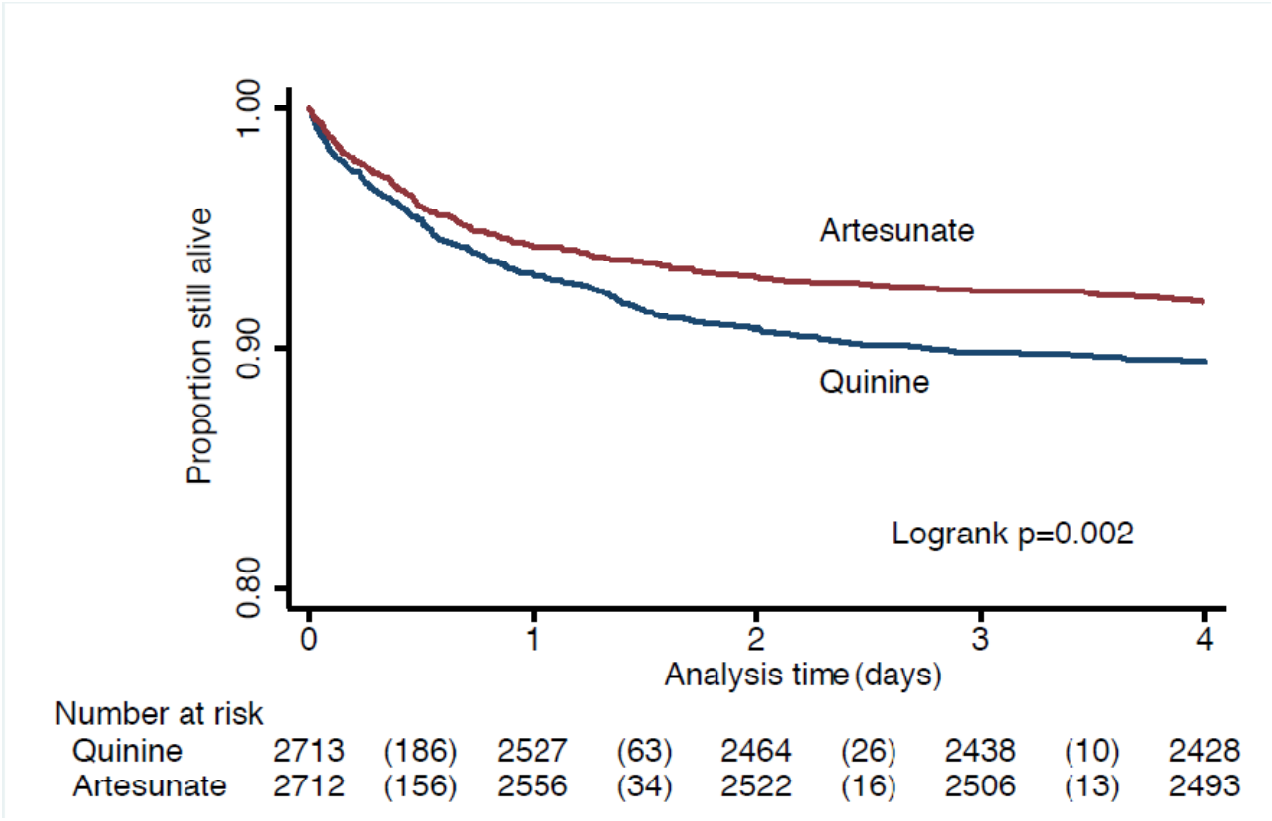
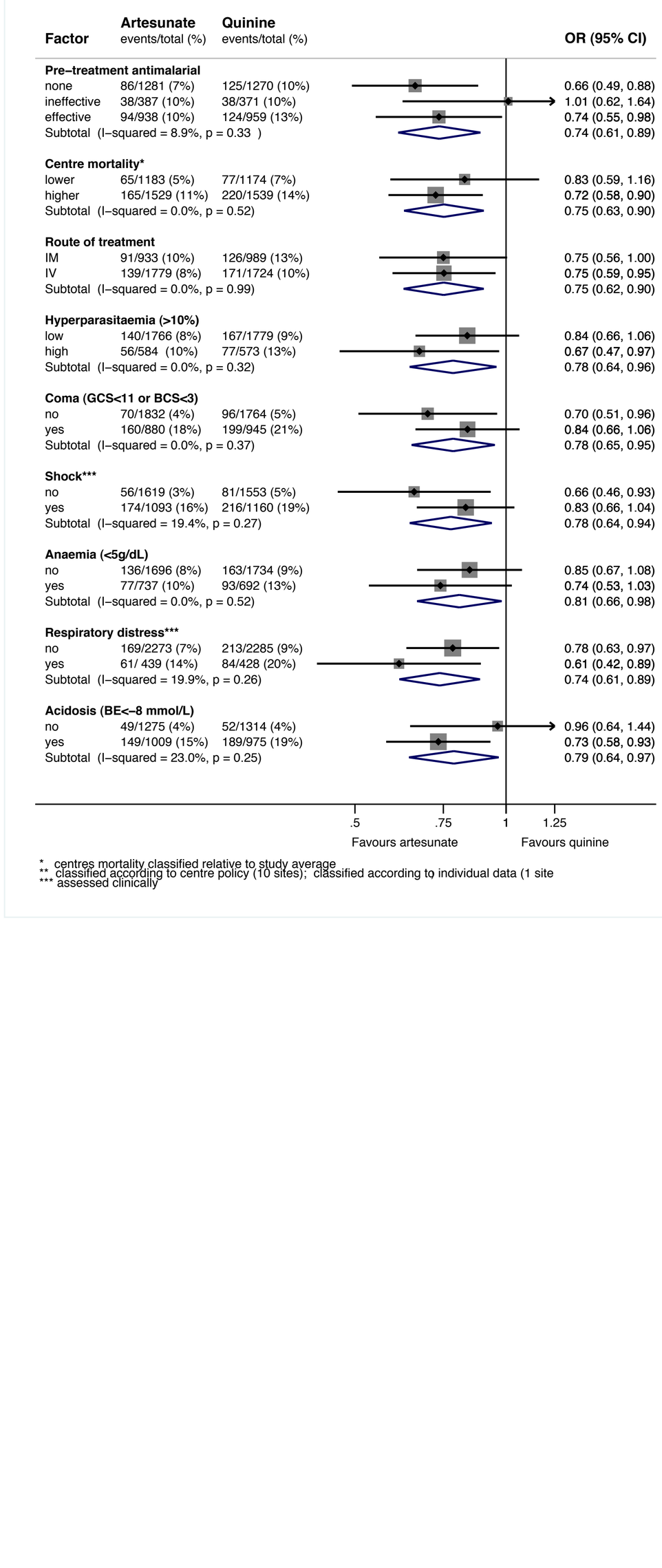

N= 11148 assessed for eligibility
N= 5723 excluded
223 insufficient clinical severity criteria
4910 negative or invalid Optimal test
180 adequate previous antimalarial treatment
316 refused consent
23 died before enrollment
25 below age or weight criteria
46 other reasons
N= 5425 enrolled
(Beira N= 663, Kilifi N= 442, Kumasi N= 436, Muheza= 921, Korogwe N= 540, Banjul N= 502, Ilorin N= 450, Ruanda N= 386, Mbarare N= 663, Kinshasa N= 422)
N= 2712 randomised to artesunate
N= 2713 randomised to quinine
N= 149
17 blood smear negative
(of which 2 also other criteria)
112 missing slide
6 died before receiving drug
7 missed early treatment dose(s)
7 unknown number of treatment doses
2 withdrawn consent
N= 161
25 blood smear negative
99 missing slide
22 died before receiving drug
4 missed early treatment dose(s)
8 unknown number of treatment doses
3 didn't fulfill entry criteria
N= 2563 analysed "per protocol"
N= 2552 analysed "per protocol"

Figure 2.



Number at risk

Quinine	2713	(186)	2527	(63)	2464	(26)	2438	(10)	2428
Artesunate	2712	(156)	2556	(34)	2522	(16)	2506	(13)	2493



* centres mortality classified relative to study average
** classified according to centre policy (10 sites); classified according to individual data (1 site
*** assessed clinically

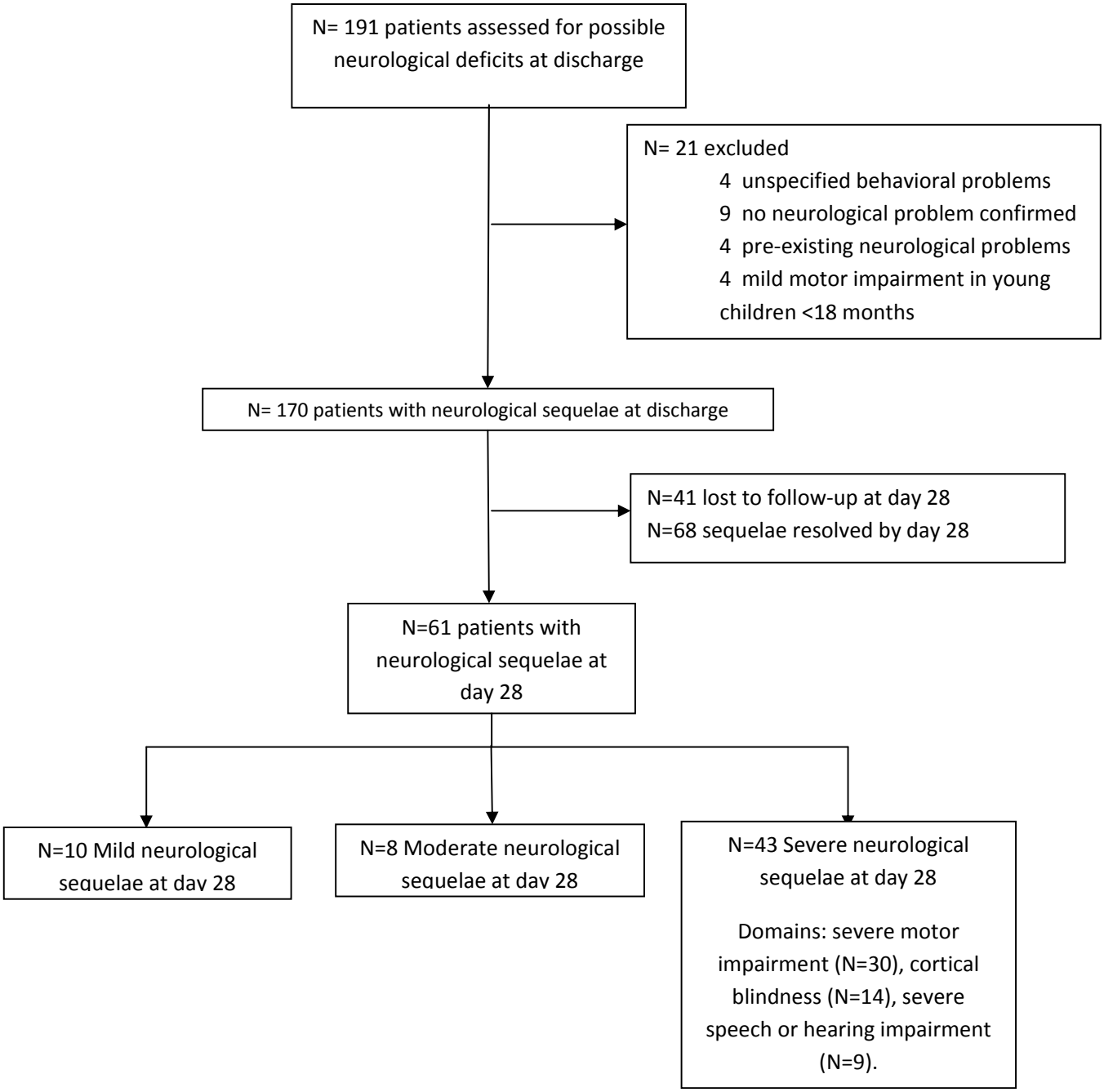
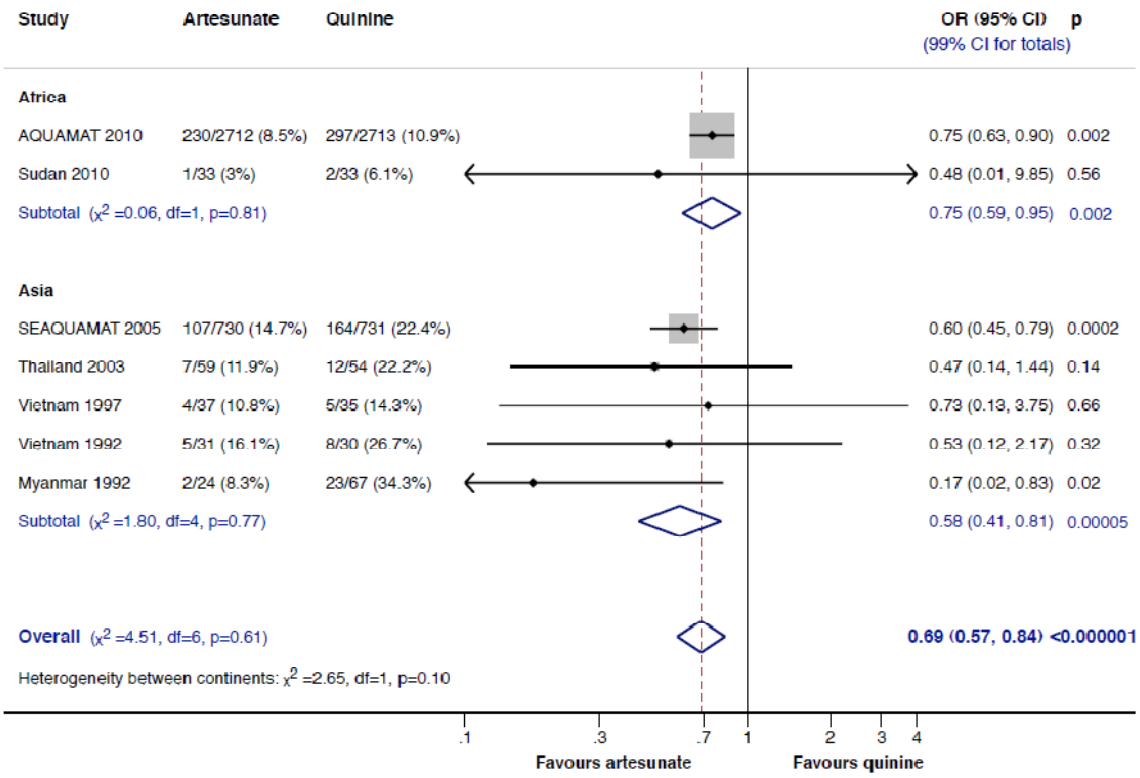

N= 191 patients assessed for possible neurological deficits at discharge
N= 21 excluded
4 unspecified behavioral problems
9 no neurological problem confirmed
4 pre-existing neurological problems
4 mild motor impairment in young children <18 months
N= 170 patients with neurological sequelae at discharge
N=41 lost to follow-up at day 28
N=68 sequelae resolved by day 28
N=61 patients with neurological sequelae at day 28
N=10 Mild neurological sequelae at day 28
N=8 Moderate neurological sequelae at day 28
N=43 Severe neurological sequelae at day 28
Domains: severe motor impairment (N=30), cortical blindness (N=14), severe speech or hearing impairment (N=9).

Figure 5.


Study
Artesunate
Quinine
OR (95% CI) p
(99% CI for totals)
Africa
AQUAMAT 2010 230/2712 (8.5%) 297/2713 (10.9%) 0.75 (0.63, 0.90) 0.002
Sudan 2010 1/33 (3%) 2/33 (6.1%) 0.48 (0.01, 9.85) 0.56
Subtotal (χ^2 =0.06, df=1, p=0.81) 0.75 (0.59, 0.95) 0.002
Asia
SEAQUAMAT 2005 107/730 (14.7%) 164/731 (22.4%) 0.60 (0.45, 0.79) 0.0002
Thailand 2003 7/59 (11.9%) 12/54 (22.2%) 0.47 (0.14, 1.44) 0.14
Vietnam 1997 4/37 (10.8%) 5/35 (14.3%) 0.73 (0.13, 3.75) 0.66
Vietnam 1992 5/31 (16.1%) 8/30 (26.7%) 0.53 (0.12, 2.17) 0.32
Myanmar 1992 2/24 (8.3%) 23/67 (34.3%) 0.17 (0.02, 0.83) 0.02
Subtotal (χ^2 =1.80, df=4, p=0.77) 0.58 (0.41, 0.81) 0.00005
Overall (χ^2 =4.51, df=6, p=0.61) 0.69 (0.57, 0.84) <0.000001
Heterogeneity between continents: χ^2 =2.65, df=1, p=0.10
.1
.3
.7
1
2
3
4
Favours artesunate
Favours quinine