Articles

Artesunate combinations for treatment of malaria: meta-analysis

*International Artemisinin Study Group**

Summary

Background Addition of artemisinin derivatives to existing drug regimens for malaria could reduce treatment failure and transmission potential. We assessed the evidence for this hypothesis from randomised controlled trials.

Methods We undertook a meta-analysis of individual patients' data from 16 randomised trials (n=5948) that studied the effects of the addition of artesunate to standard treatment of *Plasmodium falciparum* malaria. We estimated odds ratios (OR) of parasitological failure at days 14 and 28 (artesunate combination compared with standard treatment) and calculated combined summary ORs across trials using standard methods.

Findings For all trials combined, parasitological failure was lower with 3 days of artesunate at day 14 (OR 0·20, 95% CI 0·17–0·25, n=4504) and at day 28 (excluding new infections, 0·23, 0·19–0·28, n=2908; including re-infections, 0·30, 0·26–0·35, n=4332). Parasite clearance was significantly faster (rate ratio 1·98, 95% CI 1·85–2·12, n=3517) with artesunate. In participants with no gametocytes at baseline, artesunate reduced gametocyte count on day 7 (OR 0·11, 95% CI 0·09–0·15, n=2734), with larger effects at days 14 and 28. Adding artesunate for 1 day (six trials) was associated with fewer failures by day 14 (0·61, 0·48–0·77, n=1980) and day 28 (adjusted to exclude new infections 0·68, 0·53– 0·89, n=1205; unadjusted including reinfections 0·77, 0·63–0·95, n=1958). In these trials, gametocytes were reduced by day 7 (in participants with no gametocytes at baseline 0·11, 0·09–0·15, n=2734). The occurrence of serious adverse events did not differ significantly between artesunate and placebo.

Interpretation The addition of 3 days of artesunate to standard antimalarial treatments substantially reduce treatment failure, recrudescence, and gametocyte carriage.

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*Members listed at end of report

Correspondence to: Prof P Garner, International Health Research Group, Liverpool School of Tropical Medicine, Liverpool, UK (e-mail: pgarner@liv.ac.uk)

Introduction

Malaria-related morbidity and mortality are rising as a consequence of drug resistance.[1–3] Treatment policy makers aim for cost-effective regimens that keep development of antimalarial drug resistance to a minimum. A strategy that could achieve both aims is the combination of standard antimalarial drugs with a second antimalarial drug, particularly artemisinin derivatives.[4,5] Resistance in *P falciparum* malaria to artemisinin compounds has not been reported.[6] These drugs act rapidly, and kill malaria parasites that are resistant to other drugs. Their rapid elimination and high intrinsic effectiveness reduce the probability of development of resistance to them. However, if artemisinin or one of its derivatives is given alone, completion of a 7-day treatment course is needed. The objective of current artemisinin-based combination treatment is for a 3-day course to act over two asexual cycles to substantially reduce total parasite numbers, ensuring a rapid clinical response. This treatment leaves a residual maximum of less than 1×10^5 parasites in the body that a slowly eliminated combination drug can remove in the third and subsequent asexual cycles.[7] Both drugs protect each other from the emergence of resistance.

Artemisinin compounds reduce gametocyte carriage and therefore infectivity.[8,9] Thus, combination treatment might also decrease malaria transmission, especially in areas of low endemicity. Such treatment has been used for over 10 years on the Thai-Myanmar border, where there is low seasonal transmission of multidrug resistant *P falciparum*. Despite the high prevalence of mefloquine-resistant *P falciparum* before use of this regimen, combination of artesunate and mefloquine has achieved sustained high cure rates (>95%), reduced *P falciparum* transmission and the incidence of falciparum malaria, and halted the progression of resistance to mefloquine.[10]

In 1998, experts agreed that WHO and the Special Programme in Research and Training in Tropical Diseases (WHO/TDR) should co-ordinate trials to assess the safety and effects of artemisinin combination treatment, concentrating on antimalarial drugs used in Africa (chloroquine, amodiaquine, and sulfadoxine-pyrimethamine).[11] Placebo-controlled trials were set up to assess various artemisinin combinations in different countries, so that workers in malaria-control programmes could estimate the potential use of these combinations for first-line drug treatment. This standardised approach allowed a prospective analysis of individual patient data analysis.

Such analysis, in comparison with aggregate meta-analysis, allows assessment of the quality of the randomisation procedure, incorporation of updated follow-up information, assessment of uniformity of data, management of missing information, improved analysis of time-to-event outcomes, and more robust subgroup analyses. Additionally, the process also allows a balanced interpretation of results, wide endorsement of the findings,

	Artesunate in combination; randomised design				
	Chloroquine	Amodiaquine	Sulfadoxine-pyrimethamine	Mefloquine	Total
Background drug					
Trials	3	3	7	3	16
Number randomised	1000	936	3005	1007	5948*
Number analysed day 14	921	887	2817	1007	5632
Number analysed day 28	901	870	2668	1007	5446
Number on day 28 with PCR results	853	843	1801†	None‡	3497

*Uganda-Mak randomised some patients who did not subsequently develop malaria—these are excluded from this total. †Peru, Kenya-K, Uganda-Mak; and ‡Thai-1, Thai-2, Thai-3 studies did not examine PCR.

Table 1: **Total patients recruited and followed up by background drug**

clear definition of further research needed, and the ability to incorporate new results.[12] Our analysis was prospective, because we wrote the analytical protocol for the meta-analysis before the results of most of the trials were known. We aimed to measure the effects of adding an artemisinin derivative (artesunate) to existing treatment regimens for participants with acute uncomplicated *P falciparum* malaria.

Methods

Trial identification and selection

Trials were eligible for inclusion if they were randomised and compared artesunate plus a standard antimalarial drug with the standard drug alone for treatment of acute, uncomplicated *P falciparum* malaria. In addition to the WHO/TDR sponsored studies,[13–15] we systematically sought additional studies in MEDLINE and the Cochrane Central Register of Controlled Trials, using the terms malaria and artesunate. We wrote to investigators who had published trials[16–19] of artesunate for malaria and to identify further relevant studies.[16–19] If potentially relevant trials were identified, we requested data for individual patients from the original datasets. All relevant researchers were invited to collaborate in the International Artemisinin Study Group. A secretariat was responsible for daily management, and an advisory committee established for oversight of quality and methods.

Study design and endpoints

Primary outcome was parasitological failure rates by days 14 and 28.[16] We used treatment failure rather than cure for the primary outcome because the odds ratio (OR) is more informative for a small event rate. Failure by day 14 was defined as (1) development of severe malaria, or danger signs of severe disease (persistent vomiting, prostration, convulsions, or impaired consciousness), (2) parasitaemia at 48 h equal to, or more than, parasite count at day 0, (3) parasitaemia on day 3 equal to, or more than, 25% of parasite count at day 0 and fever (axillary temperature $\geq$37·5°C or rectal temperature $\geq$38·5°C), or parasitaemia on day 4 equal to, or more than, 25% of day 0 count, (4) parasitaemia on day 7, (5) initial parasite clearance by day 7 followed by recurrence by day 14, (6) an adverse event causing study withdrawal, or (7) use of further drugs with antimalarial activity between days 0 and 14. A secondary analysis assessed whether day 14 treatment failures were early—ie, defined as (1), (2), or (3).

Recurrent parasitaemia might result from recrudescence of the original infection or a newly acquired malarial infection with a new genotype.[20] We used PCR genotyping of paired blood samples to count the number of participants with recrudescent infection by day 28 (excluding reinfection). Secondary outcomes were parasitological failure by day 28 irrespective of PCR result, time to parasite clearance (the first recorded negative slide), time to fever clearance, and gametocyte carriage. We sought serious adverse events—defined as a sign, symptom, or intercurrent illness that was fatal, life threatening, or needed admission to hospital.

Addition of artesunate was compared with placebo (or with no artesunate for open-label trials) stratified by background drug in the main analysis. Data were presented with the OR for meta-analysis within individual drug groups, and across all drug groups, if the direction of effect between trials were consistent. We anticipated heterogeneity, and investigated whether this could be accounted for by: (1) background drug; (2) age; (3) whether

	Year	Concealed allocation	Masking	Background drug	
				Dose (mg/kg^{-1} day^{-1})	Days of artesunate treatment
Background drug					
Chloroquine					
Burkina Faso[13]	1999 to 2000	Yes	Double blind	10	3
Ivory Coast[13]	1999 to 2000	Yes	Double blind	10	3
Sao Tome and Principe[13]	1999 to 2000	Yes	Double blind	5	3
Amodiaquine					
Gabon[13]	1999 to 2000	Yes	Double blind	10	3
Kenya-A[14]	1999 to 2000	Yes	Double blind	10	3
Senegal[14]	1999 to 2000	Yes	Double blind	10	3
Sulfadoxine-pyrimethamine					
The Gambia[15]	1998 to 1999	Yes	Double blind	25/1·25	1 and 3
Kenya-K[13]	1999 to 2000	Yes	Double blind	25/1·25	1 and 3
Kenya-W[13]	1999 to 2000	Yes	Double blind	25/1·25	1 and 3
Malawi[13]	1999 to 2000	Yes	Double blind	25/1·25	1 and 3
Uganda-MSF[13]	1999 to 2000	Yes	Double blind	25/1·25	1 and 3
Uganda-Mak[17]	2000 to 2001	Yes	Double blind	25/1·25	3
Peru[13]	1999 to 2000	No	Open label	25/1·25	3
Mefloquine					
Thai-1[18]	1992 to 1993	Yes	Open label	25*	1
Thai-2[18]	1992 to 1993	Yes	Open label	25†	3
Thai-3[19]	1992 to 1993	Yes	Open label	25‡	3

*Artesunate and mefloquine 25 mg base/kg given together. †Artesunate 4 mg/kg on day 1, 2 mg kg^{-1} day^{-1} for 2 days, mefloquine given on second day of treatment. ‡Artesunate 4 mg/kg for 3 days, mefloquine given on second day of treatment.

Table 2: **Included trials**

	Number randomised	Number analysed day 14	Male (%)	Age (years)	Weight (kg)	Temperature on enrolment
Background drug						
Chloroquine						
Burkina Faso	300	290	53·7	1·8 (1·0)	9·4 (2·4)	38·6° (0·8)
Ivory Coast	300	266	47·3	2·2 (1·2)	10·3 (2·7)	37·7° (0·7)
Sao Tome and Principe	400	365	48·3	4·2 (2·4)	11·4 (3·1)	37·7° (1·1)
Amodiaquine						
Gabon	218	190	47·7	5·8 (2·3)	18·3° (5·1)	37·6 (1·2)
Kenya-A	398	380	46·7	2·8 (2·3)	12·0 (4·5)	38·4° (1·2)
Senegal	320	317	48·4	8·1 (2·9)	23·8 (7·9)	38·5° (0·9)
Sulfadoxine-pyrimethamine						
Gambia	598	575	51·6	4·7 (2·3)	15·1 (5·0)	37·7° (1·2)
Kenya-K	600	581	51·0	1·4 (1·1)	9·4 (3·0)	37·8° (1·2)
Kenya-W	600	567	52·8	2·6 (1·4)	10·0 (2·8)	38·1° (1·3)
Malawi	450	409	52·2	3·8 (2·5)	13·2 (4·7)	38·0° (1·3)
Peru	196	191	59·7	29·0 (14·6)	55·1 (15·9)	37·7° (1·2)
Uganda-MSF	407	376	45·7	2·2 (1·3)	11·3 (2·8)	37·8° (1·3)
Uganda-MAK	210*	118	47·5	3·0 (1·4)	..	..
Mefloquine						
Thailand-1	298	298	63·8	18·6 (13·7)	37·0 (15·8)	37·8° (1·1)
Thailand-2	349	349	56·2	15·9 (12·7)	32·2 (16·3)	37·9° (1·1)
Thailand-3	360	360	51·0	18·5 (15·0)	32·5 (15·4)	37·8° (1·1)
Total	6004	5632				

Data are mean (SD) unless otherwise indicated. *Some children who were randomised did not develop malaria.

Table 3: **Baseline characteristics of participants**

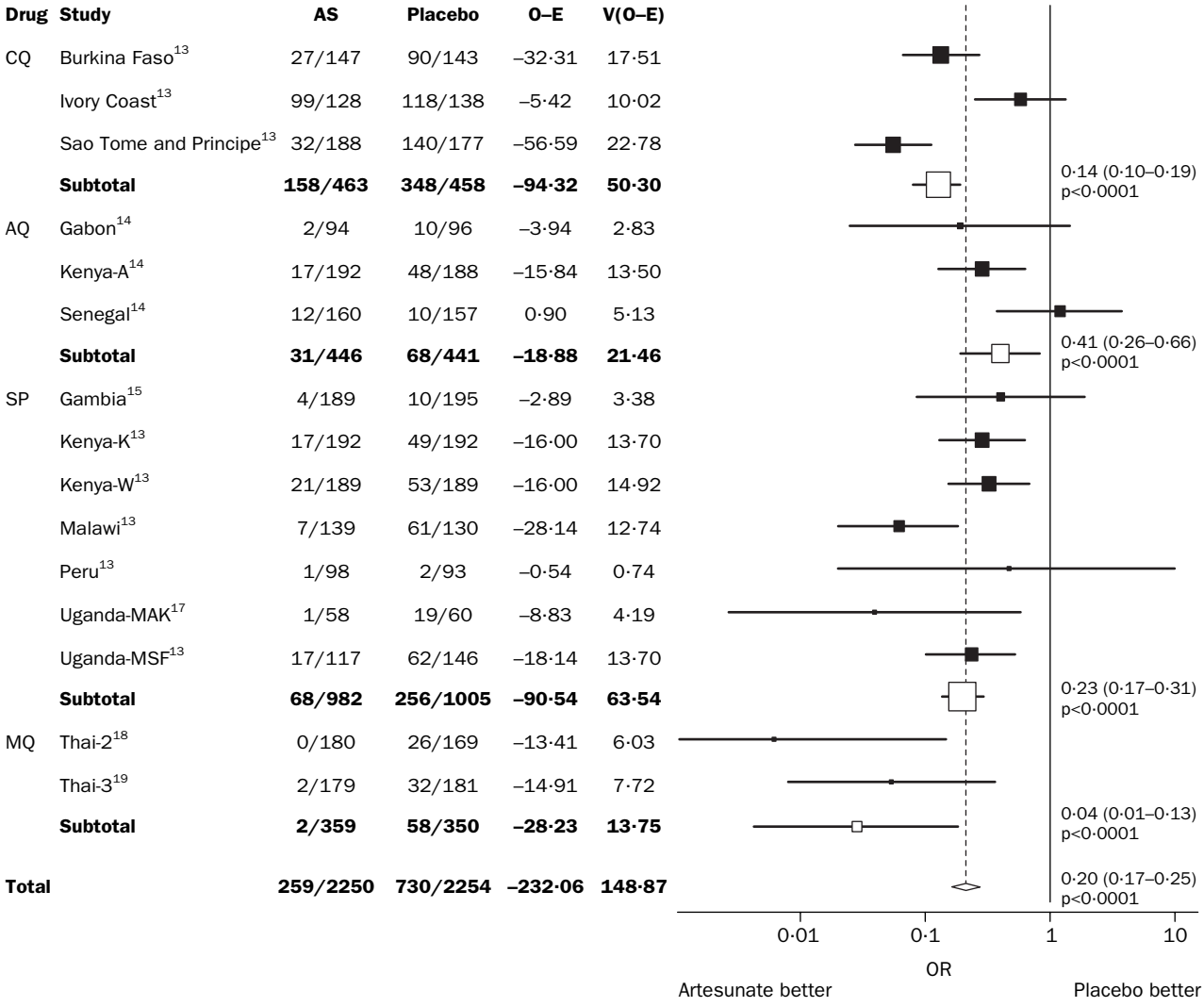


Figure 1: **Parasitological failure by day 14: 3-day artesunate *vs* placebo**

CQ=chloroquine. AQ=amodiaquine. SP=sulfadoxine-pyrimethamine. MQ=mefloquine. AS=artesunate. p<0·0001 for heterogeneity between background drugs and heterogeneity between trials.

malaria transmission was seasonal (4–6 months) or perennial; (4) baseline indices indicative of disease severity (parasite count, anaemia); and (5) overall failure rate of the background drug.[16] We considered investigating heterogeneity by regions (Africa, Asia, and South America) but decided not to since the analysis is confounded by background drug (all the mefloquine trials were in Asia; all the chloroquine and amodiaquine studies were in Africa; and all but one of the sulfadoxine-pyrimethamine trials were in Africa).

Data analysis

We assessed trial quality by adequacy of random allocation, inclusion of all eligible randomised participants in the analysis, and completeness of follow-up. We analysed randomisation by tabulating the number of participants assigned to each treatment group in weekly intervals from the time of enrolment, and by comparing baseline characteristics between the treatment groups. For the WHO/TDR trials of artesunate combinations, one protocol was used and an analytical plan designed.[21] Every trial was carefully assessed with internal checks, which identified missing and outlying data. For studies that had been published previously, we identified discrepancies and followed these up with the trial investigators. Participants were excluded from our meta-analysis if they were wrongly randomised because of: (1) a false positive slide on day 0 (initially slide-positive but later shown to be negative), (2) the presence of defining symptoms or signs of severe malaria, or (3) an additional clinically significant illness on day 0.

A substantial number of PCR results were missing because of no sample, failed PCR analysis, or equivocal PCR results. Our primary analysis for failure by day 28 excluded participants with missing PCR data (from both numerator and denominator). However, we did two further analyses for sensitivity; the first assumed that participants with missing data were treatment failures and the second assumed they were treatment successes.

	Artesunate (3 days) early failure/all day 14 failures (%)	Placebo early failure/all day 14 failures (%)	Countries
Background drug			
Chloroquine	8/463 (1·7)	87/458 (18·7)	Burkina, Ivory Coast, Sao Tome and Principe
Amodiaquine	4/446 (1)	6/441 (1·3)	Gabon, Kenya-A, Senegal
Sulfadoxine-pyrimethamine	23/982 (2·3%)	35/1005 (3·4)	Gambia, Kenya-K, Malawi, Peru, Uganda

Table 4: **Day 14 failures categorised as early**

Statistical analysis

For binary outcomes (eg, parasitological failure by day 14), we used the Peto-Mantel-Haenszel method to test for differences in these outcomes between artesunate combination treatment and standard drug alone.[22–24]

Drug	Study	AS	Placebo	O–E	V(O–E)
CQ	Burkina Faso[13]	74/145	115/142	–21·49	16·19
	Ivory Coast[13]	115/124	129/134	–2·27	3·32
	Sao Tome and Principe[13]	85/181	154/175	–36·51	19·69
	Subtotal	**274/450**	**398/451**	**–60·27**	**39·19**
AQ	Gabon[14]	14/94	28/98	–6·56	8·24
	Kenya-A[14]	57/180	108/183	–24·82	22·56
	Senegal[14]	29/159	33/156	–2·30	12·49
	Subtotal	**100/433**	**69/437**	**–33·68**	**43·29**
SP	Gambia[15]	6/187	20/193	–6·79	6·07
	Kenya-K[13]	89/192	121/189	–16·83	23·62
	Kenya-W[13]	52/189	107/189	–27·50	23·09
	Malawi[13]	41/134	99/129	–30·33	16·43
	Peru[13]	2/97	4/93	–1·06	1·46
	Uganda-MSF[13]	48/116	89/144	–13·12	16·08
	Subtotal	**238/915**	**440/937**	**–95·64**	**86·75**
MQ	Thai-2[18]	1/180	46/169	–23·24	10·19
	Thai-3[19]	9/179	57/181	–23·82	13·51
	Subtotal	**10/359**	**103/350**	**–47·06**	**23·70**
Total		**622/2157**	**1110/2175**	**–236·64**	**192·93**

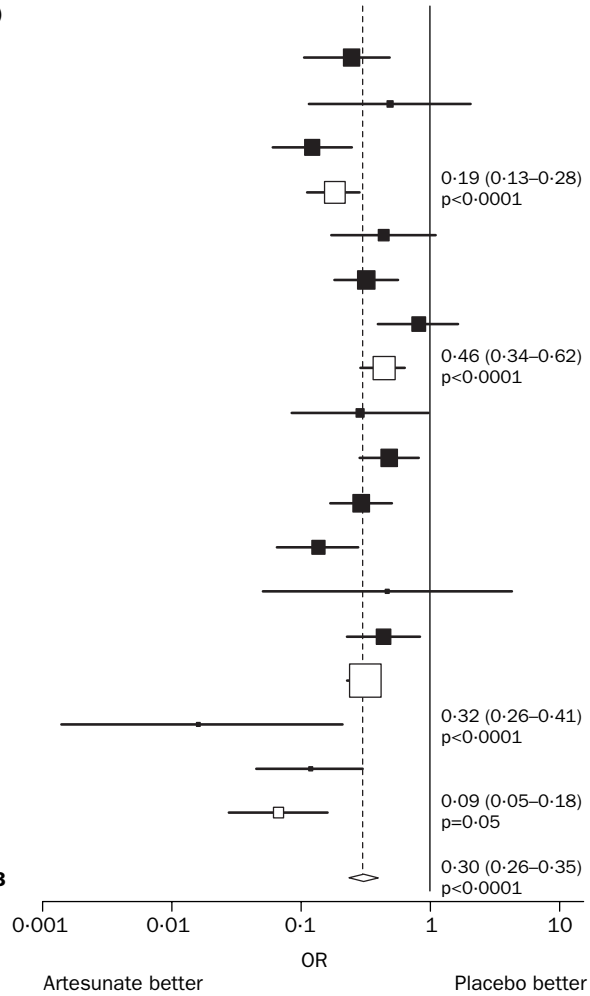
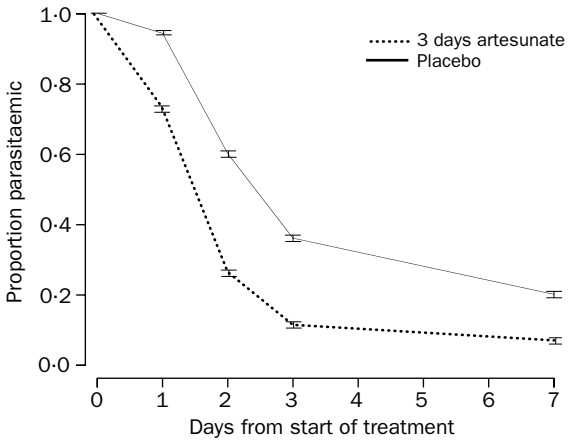


Figure 2: **Parasitological failure (including reinfections) by day 28: 3-day artesunate *vs* placebo**
p<0·0001 for heterogeneity between background drugs and heterogeneity between trials.



Numbers of patients who have not cleared their parasites

Combination	1935	1201	329	134	..	..	..	89
Standard drug	1969	1695	1176	653	..	..	..	269

Figure 3: **Survival curve to time for parasite clearance**
Vertical bars are 95% CIs.

This analysis is based on the difference (O–E) between the number of observed (O) failures in the artesunate combination treatment and the expected (E) number under the null hypothesis of no effect of the addition of artesunate to standard treatment. We calculated O–E and its variance (V) for each trial. These were then added to obtain a summary of O–E and V across all trials, and of trials within a specific drug group (defined by the background standard treatment). The value of $(O–E)^2/V$ was then referred to the χ^2 distribution with one degree of freedom.

To obtain a combined estimate of the effect of addition of artesunate to standard treatment for specific drug groups, and for all trials together, we calculated for each trial log OR of failure in the combination treatment group compared with background treatment alone. We did this using conditional logistic regression (STATA version 8). A summary OR across trials was calculated with a weighted average of trial-specific log ORs, with weights inversely proportional to their variances. Although, numerically, this summary OR is the same as the fixed effect estimate, we did not interpret this summary measure as a common OR, which is identical for all trials. As a weighted average, it can be interpreted as a typical OR in the trials included, under the assumption that the direction of the effect of addition of artesunate to background treatment is the same for all trials, but might differ in magnitude between trials.[25]

We used meta-regression to explore possible modifying effects of pre-specified factors.[26,27] For time to event outcomes (eg, time to parasite clearance), life table methods stratified by individual trials were used.[22] Data for gametocyte carriage on days 7, 14, and 28 were analysed together and then separately, for participants with and without gametocytes at baseline. Quantitative data for gametocytes were log transformed (to log [gametocyte count+1]) and summarised for every participant by the mean change from baseline, as measured by the area under the gametocytaemia time curve minus baseline to Day 28. We calculated differences in the mean of the time curve minus baseline between treatment groups for all trials and combined them using standard methods.

ORs for individual trials and meta-analysis of trials with the same background drug are presented with 99% CIs. 95% CIs were used for summary ORs across drug groups. In the graphs, each OR estimate is indicated by the corresponding square (solid within individual trials and open across groups of trials with the same background drug); horizontal lines represent 99% CIs. The sizes of the squares are directly proportional to the amount of information each trial contributes to the meta-analysis. The diamond represents the effect of artesunate across all background drugs, with the diamond's vertical axis indicating the OR, and the span the 95% CIs.

Role of funding source

The funding sources had no role in study design, data collection, data analysis, data interpretation, or in the writing of the report.

Results

27 studies were considered for inclusion; 11 did not meet the entry criteria (three were not randomised; five had different doses or timing; one used artesunate in both groups; two used different background drugs in the two groups). Sixteen trials met the inclusion criteria (table 1), and all provided data for individual patients (tables 2 and 3). 12 were done in Africa, three in Thailand, and one in Peru. 12 were placebo-controlled and double-blinded. The four trials outside Africa (Peru and three in Thailand) were open label. The background drugs were chloroquine (three trials), amodiaquine (three), sulfadoxine-pyrimethamine (seven), or mefloquine (three). The intervention group recieved standard treatment combined with artesunate. 15 investigations assessed 3 days of artesunate, of which five had an additional group of 1 day of artesunate; one assessed only the addition of 1 day of artesunate. The dose of artesunate was 4 mg per kg bodyweight daily for 1 or 3 days, except for one trial[18] (table 2).

The WHO/TDR co-ordinated studies were undertaken across Africa in countries where day 14 parasitological failure rates to the background drug were estimated to be less than 25%. The results of the trials showed that resistance was worse than anticipated. The African trials enrolled children only, whereas adults and children were recruited in Thailand and Peru. Both sexes were enrolled in all studies.

In 15 trials, investigators assessed the addition of 3 days of artesunate to background treatment in terms of failure by day 14 (figure 1). The addition of 3-day artesunate was associated with substantially lower day 14 failure rate. This effect was evident for all the background standard treatments. Figure 2 shows summary ORs for each group of background drugs (combined summary OR 0·20, 95% CI 0·17–0·25, n=4504).

There was significant heterogeneity in the size of the effect of artesunate between background drugs—eg, the estimated OR for amodiaquine was 0·41 and for mefloquine was 0·04. However, this finding did not account for most of the heterogeneity between trials ($p<0·0001$). In a meta-regression analysis, after adjusting for residual heterogeneity between trials within background drug groups, we noted a marginal effect only of background drug on the size of benefit from addition of artesunate ($p=0·09$). We therefore explored other factors to explain this heterogeneity.

To investigate whether the effect of artesunate was affected by the failure rate of background drugs, we grouped trials by overall failure rates. We used overall failure rates to avoid bias due to regression to the mean. For a failure rate of 0–10%, the OR was 0·36 (95% CI 0·21–0·63, six trials); for 11–25%, the OR was 0·28 (0·20–0·39, four studies); and for 26% and above, the OR was 0·14 (0·11–0·19, five trials). Although these ORs varied greatly by overall failure

Drug	Study	AS	Placebo	O–E	V(O–E)
CQ	Burkina Faso[13]	16/130	30/115	–8·41	9·34
	Ivory Coast[13]	16/94	10/134	1·54	5·46
	Sao Tome and Principe[13]	10/181	34/128	–15·77	9·19
	Subtotal	**42/405**	**74/318**	**–22·64**	**23·99**
AQ	Gabon[14]	3/81	8/85	–2·37	2·58
	Kenya-A[14]	8/152	24/160	–7·59	7·20
	Senegal[14]	2/152	3/150	–0·52	1·23
	Subtotal	**13/385**	**35/395**	**–10·47**	**11·01**
SP	Gambia[15]	15/152	93/154	–38·65	17·53
	Kenya-K[13]	6/166	59/161	–27·00	13·06
	Malawi[13]	5/115	42/111	–18·92	9·34
	Peru[13]	4/91	69/92	–32·30	11·03
	Uganda-MSF[13]	8/87	82/102	–33·43	11·77
	Subtotal	**38/611**	**345/620**	**–150·30**	**62·73**
Total		**93/1401**	**454/1333**	**–183·41**	**97·74**

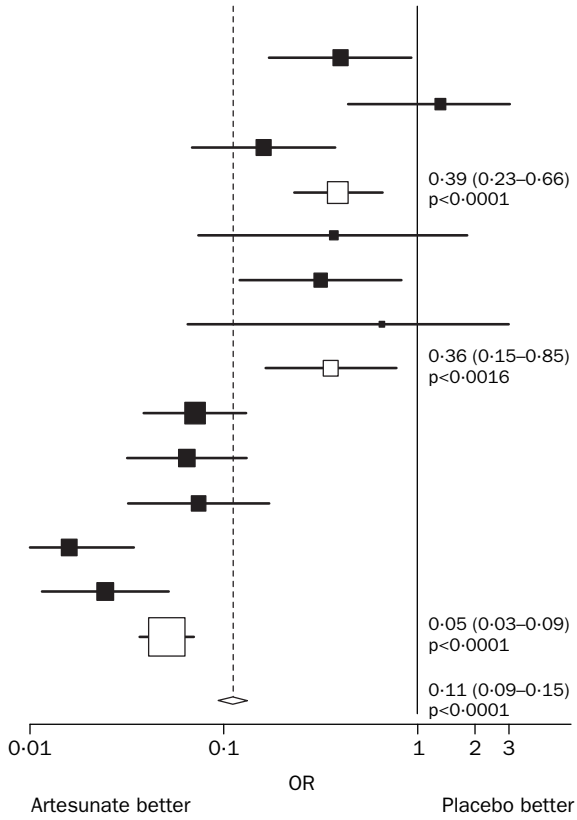


Figure 4: **Gametocytes at day 7***
p<0·0001 heterogeneity between background drugs and heterogeneity between trials. *for participants who had no gametocytes at baseline.

rate, there remained substantial residual heterogeneity between trials within groups that had equivalent overall failure rates. Even within trials of one drug in countries with similar overall failure rates, the difference in effect size was striking. For example, for chloroquine, background failure was greater than 26% in all three trial sites, with ORs varying substantially (0·13 for Burkina Faso, 0·58 for Ivory Coast, and 0·05 for Sao Tome and Principe). After allowing for residual heterogeneity between trials within groups of trials with equivalent failure rates, we recorded no relation between overall failure rate and size of the effect of adding artesunate on the day 14 assessment (p=0·7). Similar results were obtained for baseline parasitaemia.

We looked at other host and site factors: age (younger than 10 years, 10 years, and older than 10 years), intensity of malaria transmission (seasonal or perennial), and packed-cell volume. None showed any significant effect on effect size of artesunate. We assessed the effect of artesunate on early treatment failures by measuring the proportion of such failures as defined (table 4). There was a substantial

Drug	Study	AS	Placebo	O–E	V(O–E)
SP	Gambia[15]	15/187	20/193	–2·22	7·96
	Kenya-K[13]	112/191	121/189	–5·11	22·59
	Kenya-W[13]	85/187	107/189	–10·49	23·55
	Malawi[13]	90/138	99/129	–7·69	13·84
	Uganda-MSF[13]	80/113	89/144	5·69	14·31
	Subtotal	**382/816**	**436/844**	**–19·82**	**82·26**
MQ	Thai-1[18]	18/152	24/146	–3·42	9·05
	Subtotal	**18/152**	**24/146**	**–3·42**	**9·05**
Total		**400/968**	**460/990**	**–23·24**	**91·31**

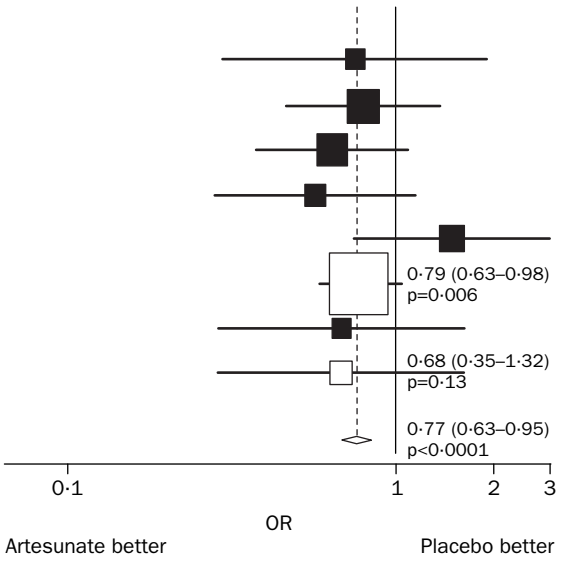


Figure 5: **Parasitological failure (including reinfections) by day 28: 1-day artesunate *vs* placebo**
p<0·0001 for heterogeneity between background drugs and heterogeneity between trials.

	Artesunate regimen	Intervention (3-day artesunate)	Control
Background drug			
Chloroquine			
Burkina Faso	3-day	Anaemia (4)* Convulsion (2)*	Convulsion (3)* Death (1)†
Ivory coast	3-day	..	..
Sao Tome and Principe	3-day	Convulsions (3)* Death (1)‡	Convulsions (16)* Convulsion + vomiting (1)* Coma (1)
Amodiaquine			
Gabon	3-day	Asthma (1)	Convulsion (1)* Vomiting (1)* Gastroenteritis (1)
Kenya-A	3-day	Convulsion (1)* Pneumonia + meningitis (1) Convulsion + anaemia (1)	Convulsion (1)* Death (1) § Pneumonia (1) Anaemia (1)
Senegal	3-day	Convulsion (1)	..
Sulfadoxine-pyrimethamine			
Gambia	3-day	Convulsion (1)*	Death (1)¶ Severe anaemia (2)*
	1-day	Convulsion (1)* Anaemia (1)*	..
Kenya-K	3-day	Death (1)|| Vomiting (1)* Severe anaemia (1)	..
Kenya-W	3-day	..	..
Malawi	3-day	Convulsion (2)* Bacterial sepsis (2)	..
	1-day	Convulsion 1 Suspected bacterial sepsis (1)*	..
Peru	3-day	..	..
Uganda-MSF	3-day	Convulsion (4)*	Convulsion (1)*
	1-day	Convulsion (1)*	..
Uganda-MAK	3-day	..	..

*Treatment discontinued. Causes of death: †acute toxic state of unknown cause; ‡severe malaria; §acute respiratory distress of unknown cause; ¶severe malaria; ||pneumonia

Table 5: **Serious adverse effects events: clinical details by study**

reduction in early treatment failures with chloroquine (OR 0·07, 95% CI 0·04–0·16) but no significant effect with amodiaquine (0·66, 0·18–2·34), or sulfadoxine and pyrimethamine (0·66, 0·39–1·13).

Ten trials provided data for day 28 failure with paired PCR samples to exclude reinfection. There remained a consistent advantage for adding 3 days of artesunate to the background drug (0·23, 0·19–0·28, n=2908; analysis excluding participants with missing PCR data). Intervention and control groups were similar in the proportion of participants with missing data. In the sensitivity analysis that counted missing PCR values as treatment failures, the estimate was similar (0·27, 0·22–0·32, n=3055). Similar results were obtained when missing PCR values were treated as treatment successes (0·24, 0·20–0·29, n=3055).

14 trials (n=4332) provided data for day 28 parasitological failure rate (ie, recrudescent and new infections combined). The results were much the same as adjusted values (0·30, 0·26–0·35, figure 2). 12 with background treatment chloroquine (three), amodiaquine (three), and sulfadoxine-pyrimethamine (six) contributed to this meta-analysis (n=3904). In each study, the addition of artesunate was associated with shorter parasite clearance times (figure 3). For all drug groups combined, parasites were cleared twice as fast with 3 days of artesunate compared with background treatment alone (rate ratio 1·98, 95% CI 1·85–2·12). The median parasite clearance time of the participants on artesunate was about a day shorter than that obtained with standard treatment (1·4 days for artesunate, 2·2 days for placebo, p<0·0001).

11 trials with background treatment of chloroquine (three), amodiaquine (three), sulfadoxine-pyrimethamine (five) contributed data to the analysis of fever clearance (n=2812). Clearance time was significantly shorter with artesunate than without (hazard ratio 1·15, 95% CI 1·06 to 1·25, p=0·001).

Gametocytaemia was measured on days 0, 7, 14, and 28 in all investigations except for three. 3163 (86%) participants did not have gametocytes at baseline. 3 days of artesunate reduced the number of participants with gametocytaemia at day 7. The effect was most pronounced for day 7 carriage, sulfadoxine-pyrimethamine group (figure 4). The summary OR was 0·11 (95% CI 0·09–0·15) for the presence of gametocytes at day 7 (3-day artesunate compared with placebo), in participants with no gametocyte at baseline (2734 participants from eleven trials). The effect of 3 days of artesunate was even more striking at day 14 (OR 0·06, 95% CI 0·04–0·09, n=2434) and day 28 (0·04, 0·02–0·08, n=1775).

To summarise transmission potential we calculated the mean change from baseline in log gametocyte count (measured by the area under the gametocyte count-time curve) in all participants irrespective of gametocyte count at baseline. This mean is a weighted average—which accounts for gametocyte assessment not being done at equally spaced timepoints. There was a mean drop from baseline in log gametocyte count of 0·158 in the artesunate group compared with a mean increase of 0·458 in the placebo group, a difference of –0·616 in the mean change (95% CI –0·698 to –0·534). This difference is equivalent to a decrease of 46% (95% CI 41–50) in the geometric mean of gametocyte count curve of artesunate compared with placebo.

Six trials (five with sulfadoxine-pyrimethamine as the background drug, and one with mefloquine) also tested the effect of adding 1 day of artesunate to standard antimalarial treatment. There was a significant reduction in the failure rate at day 14 (OR 0·61, 95% CI 0·48–0·77, n=1980) and day 28 (0·77, 0·63–0·95, n=1958, figure 5), but the effect size was less striking than with 3 days of artesunate.

In four of the five sulfadoxine and pyrimethamine studies, investigators also obtained PCR genotyping. In the meta-analysis of these trials excluding reinfection, we analysed the results by managing the missing values in the three ways described. In the analysis excluding missing values, the OR (1-day artesunate compared with no artesunate) was 0·68 (0·53–0·89, n=1205); with missing values as failures, the OR was 0·72 (0·56–0·92, n=1285); with missing values as successes, the OR was 0·69 (0·54–0·90, n=1285). 1 day of artesunate reduced gametocytes for participants with no gametocytes at baseline for day 7 (OR 0·12, 95% CI 0·08–0·16, n=1051).

Five trials of sulfadoxine-pyrimethamine provided data that were analysed for direct comparison of artesunate treatment for 1 and 3 days. The 3-day regimen was better for parasitological failures by day 14 (0·37, 0·27–0·51, n=1656) and day 28 (0·40, 0·32–0·51, n=1634). In data sets with PCR results, exclusion of reinfections gave similar results: OR 0·32, 0·24–0·43 (participants with missing PCR excluded); 0·36, 0·27–0·47 (participants with missing PCR assumed to be failures); 0·34, 0·25–0·46 (participants with missing PCR assumed to be successes).

In participants who had no gametocytes at day 0, artesunate for 3 days was significantly better than 1-day artesunate in reducing gametocytes at day 7 (OR 0·49, 95% CI: 0·32 to 0·76, n=1043), and day 14 (0·52, 0·27–0·99, n=996). We detected no significant difference in effect

between the two regimens at day 28, but the 95% CIs were wide and are therefore consistent with a substantial advantage for 3-day compared with 1-day artesunate (gametocytes negative at baseline: OR 0·25, 95% CI 0·01–1·98, n=742).

The average drop from baseline in log gametocyte count in all participants, irrespective of gametocytes at baseline, was 0·232 in the 3-day artesunate group, compared with 0·121 in the 1-day group, a difference of 0·111 (0·002–0·218). This is equivalent to a decrease of 10% (0–20) in the geometric mean of gametocyte count.

The frequency of serious adverse events did not differ by much between groups who received 3 days of artesunate compared with those who received placebo, across trials that systematically recorded this information. There were five deaths; two in the combination group and three in the placebo group. All were in African children and none was regarded as related to study drugs. Investigators reported 20 participants who had convulsions in the Sao Tome and Principe trial; 17 in the controls who received chloroquine alone, and three in the group who also received artesunate (table 5).

Discussion

Our results for parasite failure at day 14 showed a consistent, large effect of adding 3 days of artesunate treatment to any of the existing drug regimens, irrespective of background treatment. The overall rate of treatment failure with standard treatment by day 14 was about 32% in the 15 trials analysed. This rate is higher than was anticipated at the planning stage of these investigations. A summary OR of 0·20 for the addition of artesunate is equivalent (with this overall failure) to a relative risk of 0·27, which translates to an average reduction of 73% in the absolute risk of failure. However, high level of consistency in the direction of the effect across background drugs is noticeable, and the effect is quantitatively important. However, the magnitude of the effect of artesunate varied significantly between trials.

We explored the heterogeneity in effect size by assessing possible sources in different circumstances. Although the effect of artesunate varied greatly between background drugs, overall failure rates at study sites, and baseline parasitaemia, there remains significant heterogeneity between trials with the same background drug and equivalent overall failure rate.

The trials we analysed had low numbers of participants who had been excluded or lost to follow up (of number randomised, 5% by day 14, and 6% by day 28). The design of this meta-analysis allowed us to treat losses to follow-up in the same way across all investigations, and to investigate factors that might have affected the presence or size of an effect with artesunate.

Decisions to change national antimalarial treatment policies have substantial health, implementation, and cost implications, and need to be based on reliable data. There is an increasing trend to change to artemisinin-based combinations failing first line treatment. The rationale for choosing such combination treatments is to ensure rapid and reliable cure, slow the speed at which resistance develops, and, potentially, in low transmission settings, reduce the incidence of malaria.

In practice, for artemisinin-based combination treatments to lead to high cure rates, reduce transmission, and provide mutual protection against resistance, the background drug needs to be highly effective. Unfortunately, high levels of drug resistance in Africa meant that, even with the benefit conferred by the artesunate combinations, cure rates were often unsatisfactory. The combination of sulfadoxine pyrimethamine and artesunate was effective in The Gambia in West Africa, but not in East Africa, where levels of resistance were high. Amodiaquine combinations were better in resistant areas, but cure rates were still below 90%. Chloroquine combinations were unsatisfactory because of high levels of resistance in the West African trial sites. Artemisinin derivatives might improve the effectiveness of drugs that are failing such as mefloquine in Thailand, and chloroquine in the trials we analysed. However, this improvement cannot be relied upon, and drugs that are clinically effective should be used.

As drug resistance worsens, an increasing proportion of treatment failures arise. Initially, these are seen weeks after treatment, but the interval progressively shortens until early failures with an increasing risk of death are recorded. The aim of treatment in uncomplicated falciparum malaria is to prevent progression towards severe infection, and to ensure rapid and reliable cure. Artemisinin combination treatments were shown in this analysis to reduce early treatment failures with chloroquine when failure rate of the background drug was high. Additionally, combination treatments with slowly eliminated antimalarials might reduce the likelihood of subsequent episodes of malaria, which Dorsey and colleagues[17] have shown through a longitudinal design with sulfadoxine-pyrimethamine, and amodiaquine, but this was not explored in this meta-analysis.

There has been some concern that the effects of adding artesunate to existing antimalarials will not be obvious at day 28 because of reinfection in areas where transmission is high. Indeed, our protocol defined a primary outcome of the meta-analysis as day 28 parasitological failure, with reinfections (estimated by PCR).[16] PCR is regarded as useful in differentiation of whether recurrent parasitaemia is likely to represent recrudescence of the existing infection or reinfection by a new parasite.[20] This differentiation, however, proved difficult, since a large number of PCR results were missing. However, ORs of all infections (recrudescence plus new infections) were very similar to the estimate from the PCR-corrected analysis, irrespective of whether missing values were treated as missing, treatment failures, or treatment successes.

We assessed serious adverse events irrespective of their relation to study drug. These were mostly malaria related or due to intercurrent illness. Artesunate did not increase the frequency of severe adverse effects. Indeed, in Sao Tome and Principe, where chloroquine failure rates were high, artesunate was associated with fewer convulsions. No serious adverse events were clearly attributable to artesunate.

Reduction of malaria transmission is an important consideration for malaria-control programmes. Artesunate greatly lessened gametocyte carriage in all drug groups. The effect of artesunate was especially noticeable with sulfadoxine-pyrimethamine, which is associated with increased gametocyte carriage. Fall in gametocyte carriage is the probable explanation for decreased *P falciparum* transmission in western Thailand after the systematic use of artesunate-mefloquine.[10] A similar effect cannot be assumed for populations subjected to intense malaria transmission, in which some gametocyte carriage develops from infections that are asymptomatic and therefore unlikely to be treated with artemisinin-based combination treatments.

Antimalarial drug resistance compromises treatment effectiveness in most countries where malaria is endemic, and morbidity and mortality rates are rising. Policy change is recommended when failure rates exceed 25%, but by this stage, resistance is already well advanced. In many countries, failure rates exceed 25% already. Resistance

therefore compromises effectiveness of artemisinin-based combination treatments using the failing drugs as partners. If the partner drug is effective in a particular region, then the benefit of addition of artesunate will be small, which might make the strategy unappealing to policymakers. But this is the optimum setting in which to use such combination treatments to prevent the emergence of resistance, and to ensure longlasting effectiveness. Many potential artemisinin-based combination treatments are now either available or being developed, and include fixed combinations with lumefantrine, mefloquine, piperaquine, pyronaridine, and chlorproguanil-dapsone. These combinations are expected to achieve cure rates well over 90%.

Although high cure rates are clearly desirable, combination treatments are costly: the factory price is about US$1·0 for a treatment course of artesunate for an adult, and a blister pack of artesunate and sulfadoxine-pyrimethamine, or artesunate and amodiaquine costs US$1·2–1·8. These prices compare with US$0·15 for chloroquine, US$0·25 for sulfadoxine-pyrimethamine, and US$2·4 for artemether-lumefantrine (at present the only available fixed-dose coformulation). Strategies allowing resource-poor countries to purchase these new effective drugs are needed. One approach to reduce costs is to restrict combination treatment use to parasitologically proven falciparum malaria, but then the cost and difficulties of diagnosis must be accounted for. The Global Fund is a potential source of support for countries to purchase such treatment.

Obtaining money to purchase these drugs is a major obstacle. Additionally, funds for research into the best use of these compounds will also be necessary. Investigations will be needed to test specific strategies to enhance use of and adherence to 3-day combination regimens, to monitor their safety profile in widespread use, and to assess the costs, training, and organisational implications of introduction of such treatment, and the short and long term benefits.

International Artemisinin Study Group
M Adjuik, P Agnamey, A Babiker, J Baptista, S Borrmann, P Brasseur, P Carnevale, M Cisse, R Collins, U D'Alessandro, N Day, W de Boom, T Doherty, G Dorsey, P Garner, S Gikunda, V Gil, B Greenwood, J P Guthmann, M C Henry, M R Kamya, P G Kremsner, E Konaté, S Krishna, D Lalloo, P Lange, M Loolpapit, G Malenga, W Marquino, K Marsh, P Milligan, M Molyneux, K Mugittu, J Niangue, F Nosten, F Ntoumi, C Obonyo, F Ochieng, P Olliaro, A J Oloo, L Osorio, L Pinoges, G Priotto, P J Rosenthal, T Ruebush, J Simpson, S Sirima, E Some, W Taylor, F ter Kuile, A Tiono, L von Seidlein, B Watkins, N White (chair).
Advisory Group—R Collins (chair), N Day, D Lalloo, J Simpson.
Writing Committee and Secretariat—M Adjuik, A Babiker, P Garner, P Olliaro, W Taylor, N White.

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