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N-Acetylcysteine as adjunctive treatment in severe malaria: A randomized double blinded placebo controlled clinical trial

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Abstract

Objective—Markers of oxidative stress are reported to be increased in severe malaria. It has been suggested that the antioxidant N-acetylcysteine (NAC) may be beneficial in treatment. We studied the efficacy and safety of parenteral N-acetylcysteine as an adjunct to artesunate treatment of severe falciparum malaria.

Design—A randomized double-blind placebo controlled trial on the use of high dose intravenous NAC as adjunctive treatment to artesunate.

Setting—A provincial hospital in Western Thailand and a tertiary referral hospital in Chittagong, Bangladesh.

Patients—One hundred and eight adult patients with severe falciparum malaria.

Interventions—Patients were randomized to receive N-acetylcysteine or placebo as adjunctive treatment to intravenous artesunate.

Measurements and main results—A total of 56 patients were treated with NAC and 52 received placebo. NAC had no significant effect on mortality, lactate clearance times ($p=0.74$) or coma recovery times ($p=0.46$). Parasite clearance time was increased from 30h (range 6h to 144h) to 36h (range 6h to 120h) ($p=0.03$), but this could be explained by differences in admission parasitemia. Urinary F_2 -isoprostane metabolites, measured as a marker of oxidative stress, were increased in severe malaria compared to patients with uncomplicated malaria and healthy volunteers. Admission red cell rigidity correlated with mortality, but did not improve with NAC.

Conclusion—Systemic oxidative stress is increased in severe malaria. Treatment with N-acetylcysteine had no effect on outcome in patients with severe falciparum malaria in this setting.

Keywords

N-Acetylcysteine; Severe malaria; Adjunctive treatment

INTRODUCTION

The introduction of the potent and rapidly acting antimalarial drug artesunate has been a significant advance in the treatment of severe malaria, decreasing the case fatality rate in adult severe malaria by 35% compared to quinine (1). By contrast, of the many other interventions tried, none have been shown to lower mortality.

Severe falciparum malaria has been associated with an increase in oxidative stress, in relation with disease severity (2-4). Possible origins of reactive oxygen species are malaria parasites or host white cells (3-6). Although reactive oxygen species may be important for parasite clearance (2), they may be harmful to the host through its damaging effects on various cells and a reduction in erythrocyte deformability (7). Rigidity of erythrocytes reduces their life span and is thought to contribute to impaired microcirculatory flow, in addition to sequestration of parasitized erythrocytes (8). Severity of anemia correlates with measures of oxidative damage to the erythrocyte membrane in pediatric severe malaria (9).

N-acetylcysteine (NAC) is a widely used, safe and well tolerated anti-oxidant. It is the main treatment for acute paracetamol poisoning, acting through direct scavenging of free radicals and replenishment of glutathione and cysteine. It also enhances clearance of peroxides through glutathione peroxidase and related enzymes. NAC has also been shown to improve microcirculatory blood flow in acute liver failure (10). NAC may be beneficial in severe malaria through enhancing scavenging of free radicals and reducing expression of endothelial ligands (11). Red cell deformability can be improved by NAC in an *in vitro* test system (12). A pilot study in Western Thailand showed that intravenous N-acetylcysteine was associated with a significantly shorter time to normalization of plasma lactate and Glasgow Coma Score (GCS) in patients with malaria treated with quinine (13). Another study in Thailand confirmed the safety of NAC as adjunctive treatment to artesunate in severe malaria (14). However, N-acetylcysteine was shown to partly antagonize the antimalarial action of artesunate *in vitro* (15). The antimalarial effect of the artemisinin derivatives depends on the unique endoperoxide bridge within the 1,2,4-trioxane nucleus, and is mediated by the formation of free radicals upon cleavage of the endoperoxide (16).

We hypothesized that NAC, as anti-oxidant adjunctive treatment, can decrease harmful oxidative stress in severe malaria, and can have a beneficial effect on outcome parameters related to prognosis. We conducted a randomized, double-blind, placebo-controlled clinical trial, on the effect of N-acetylcysteine as adjunctive treatment to artesunate in adult patients with severe falciparum malaria. Primary endpoints were the effects on lactate clearance time, coma recovery time, and parasite clearance time.

PATIENTS AND METHODS

Study site and patients

The study was conducted at Chittagong Medical College Hospital (CMCH), Chittagong, Bangladesh and Mae Sot General Hospital, Tak, Thailand, from May 2003 to October 2005. CMCH is a 1,000 bed teaching hospital with facilities for oxygen therapy and blood transfusion. The 250 bed provincial hospital in Mae Sot has I.C.U. facilities including hemodialysis and mechanical ventilation. Malaria transmission is seasonal and of low intensity in both locations (17-18). Ethical clearance was obtained from the Ministries of

Health in Thailand and Bangladesh, and from the Oxford Tropical Medicine Ethical Committee (OXTREC). ISRCTN registration number ISRCTN20156397.

Adults patients (≥ 16 y/o) with slide-confirmed severe malaria according to modified WHO criteria (19) were recruited, provided written informed consent was obtained from the attending relative. Criteria for severe malaria included coma (GCS < 11), acute respiratory distress syndrome (ARDS), repeated convulsions, severe anemia or jaundice (hematocrit $< 20\%$, bilirubin > 3.0 mg/dl, combined with parasite counts $> 100,000/\mu\text{L}$), renal failure (serum creatinine > 3 mg/dl), hypoglycemia (blood glucose < 40 mg/dl), shock (systolic blood pressure < 80 mm Hg with cool extremities), hyperparasitemia (peripheral asexual stage parasitemia $> 10\%$), hyperlactatemia (venous plasma lactate > 4 mmol/l), or acidemia (venous plasma bicarbonate < 15 mmol/l). Pregnancy, breast feeding, a history suggesting asthma or hypersensitivity to NAC, a wheeze on auscultation on admission, or previous treatment with lactate containing intravenous fluids were exclusion criteria.

Since plasma lactate concentrations have a strong prognostic significance in severe malaria (20), we powered the study to demonstrate an increase in the proportion of patients with venous plasma lactate levels < 2 mmol/L at 24h from 20% to 50% with a significance level (α) of 0.05 and a power ($1-\beta$) of 0.80. Based on this, we required at least 90 patients in the study. The primary endpoint was chosen to inform the decision whether to expand to a larger trial with mortality as endpoint.

Drug treatment

Patients were randomly assigned to either NAC or matching placebo as adjunctive treatment to parenteral artesunate (Guilin No. 2 Pharmaceutical Factory, Guangxi, People's Republic of China). Computerized randomization was balanced in blocks of 40, and allocation codes were kept in sealed envelopes. Patients, physicians and nurses were masked to the treatment assigned. NAC was obtained as 10 ml ampoules containing 20% w/v N-acetylcysteine from Medeva, Celltech Group, Berkshire, UK, who also supplied matching ampoules containing placebo. NAC was given intravenously in the standard regime used in paracetamol intoxication, i.e. 150 mg/kg in 200 ml 5% dextrose water (5% DW) over 15 minutes, followed by 50 mg/kg in 500 ml 5% DW over 4 hours, then 100 mg/kg in 1000 ml 5% DW over 16 hours. Since NAC has a distinctive odor, treating doctors were not involved in the preparation of the infusion. The saline placebo was given in 5% DW. The first dose of NAC or placebo was started two hours after starting intravenous artesunate. The standard dosing regimen for artesunate was used (1). Supportive treatments were in accordance with World Health Organization (21) and local hospital guidelines, but availability of renal replacement therapy and mechanical ventilation was limited in the hospital in Chittagong.

Study procedures

On admission, a full history and examination were carried out. Venous blood samples were obtained for hemoglobin, hematocrit, parasitemia, platelet count, white cell count, plasma lactate levels, glucose levels and full biochemistry. Parasite counts and lactate levels were assessed every 6 hours. Red cell deformability was measured at a shear stress of 1.7 Pa on admission, 6 hrs after admission and then daily until discharge using a Laser-assisted Optical Rotational Cell Analyser (LORCA) (8). Lactate concentrations were measured from stored plasma samples (-30°C) with YSI 2300 STAT PLUS glucose/L-lactate version 250D. Urine F_2 -isoprostane metabolites (F_2 -isoP-M) levels were used as a measure of oxidative stress and assayed on admission and daily until discharge or death. Urine was collected from the urinary catheter and stored without preservative at -80°C or in liquid nitrogen. Urinary concentration of F_2 -isoP-M was measured using a high-performance liquid chromatography method coupled to electrospray ionization—tandem mass spectrometry

(LC-MS/MS). The urinary content of F₂-isoprostanes metabolites was corrected for renal function by calculating the ratio with creatinine clearance with the formula (22-23):

$$[F_2\text{-isoP-M}]_{\text{corrected}} = [F_2\text{-isoP-M}]_{\text{urine}} \times [\text{creatinine}]_{\text{plasma}} / [\text{creatinine}]_{\text{urine}}$$

Excretion of prostanoids (like F₂-isoP-M) is decreased in parallel with creatinine clearance, as shown in patients with liver disease (24). Vital signs, oxygen saturation, conscious level (GCS), fluid input and output were recorded 6 hourly until recovery. Parasite clearance time was defined as the interval between start of treatment and the first of two sequential negative thick films. PC50 is the time to 50% and PC90 to 90% reduction in parasite density. Parasite reduction ratios at 24 h (PRR24) and 48 h (PRR48) were defined as the ratio of the parasite count at admission to that at 24 or 48 h (25). Fever clearance time was the time to a tympanic measured temperature below 37.5°C. Coma recovery time was measured from the start of antimalarial treatment to the time the GCS score reached 15. Time to normal plasma lactate level was the time to a decrease in plasma concentrations <2 mmol/L (upper limit of normal value). Follow up for all patients was from admission until discharge or death.

Primary outcome measures were lactate clearance time, coma recovery time and parasite clearance time. Secondary outcome measures included fever clearance time, mortality, other parasite clearance measures (PC50, PC90, PRR24 and PRR48), change in red cell deformability and measures of oxidative stress, and the frequency of adverse events.

Statistical analysis

Analysis was performed by SPSS software (version 11.0) and STATA (version 9). Data were log transformed to obtain a normal distribution, if indicated. Normally distributed data were compared using Student's t-test and non—normally distributed data were compared using t-test for geometric means or the Mann-Whitney U test for non-paired nonparametric data or the Wilcoxon Signed Rank test for paired nonparametric data. Categorical data were compared by Pearson's chi-squared test and Fisher's exact test. Multiple groups were compared by ANOVA, and comparisons between individual groups were corrected for multiple comparisons (Bonferroni). Univariate correlations were checked with Pearson's correlation coefficient if the data were normally distributed or could be transformed to a normal distribution. Kaplan-Meier plots were constructed for time dependent parameters. For comparison between treatment groups (Wilcoxon-Breslow test) the lactate clearance times and coma recovery times in patients who died before clearance or recovery were set as infinite, since failure to clear lactate or recover from coma has strong prognostic significance for death. To assess whether treatment had any effect on the time to lactate or parasite clearance and coma recovery times, Cox regression analysis was performed, while adjusting for [log] initial parasitemia and bilirubin, since these were significantly different between the two groups at baseline. To determine whether treatment was associated with the risk of dying, a multiple logistic regression analysis was performed with mortality as the dependent variable and allocation to NAC or placebo as the independent variable. The model was also adjusted for presenting severity symptoms.

RESULTS

108 patients were enrolled in the study, 23 in Mae Sot General Hospital and 85 in Chittagong Medical College Hospital. Of these, 56 patients (13 at Mae Sot) were randomized to receive NAC and 52 (10 at Mae Sot) received placebo.

Baseline characteristics

Baseline characteristics are summarized in tables 1 and 2. Except for differences in admission parasitemia and plasma bilirubin concentration, the treatment groups were well matched.

Lactate clearance time

Elevated admission plasma lactate concentrations (>2 mmol/L) was present in 99/108 (92%) patients. Median lactate clearance time was 30 in the NAC treated and 42 hours in the placebo group (range 6 to 96 hours for NAC and 6 to 144 hours for placebo, excluding expired patients). The Kaplan-Meier plot for lactate clearance time (figure 1a), shows no difference between treatment groups (Wilcoxon-Breslow test, $p=0.74$), also after adjustment for [log] admission parasitemia and bilirubin (Hazard ratio (HR) 0.98, 95% CI 0.60 to 1.62, $p=0.95$).

Coma recovery time

On admission 91/108 (84%) patients had impaired consciousness ($GCS < 15$) of which 78 (72%) were in coma ($GCS < 11$). Median coma recovery time was 72 hours in NAC treated patients and 96 hours in the placebo group (range 6 to 120 hours in NAC and 6 to 360 hours in the placebo group, excluding the patients who died). Kaplan-Meier analysis, where the coma recovery time for the fatal cases was regarded as infinite, showed no difference between treatment groups (figure 1b, Wilcoxon-Breslow test, $p=0.46$). Treatment had no effect on coma recovery time after adjustment for [log] admission parasitemia and bilirubin (HR 1.05, 95% CI 0.58 to 1.88, $p=0.88$).

Parasite clearance time

Using Kaplan Meier analysis (figure 1c) parasite clearance was faster in patients receiving placebo (Wilcoxon-Breslow $p=0.03$). Parasite clearance time, censoring the fatal cases at the time of death, was 36 hours (range 6 to 120) in the NAC treated patients and 30 hours (range 6 to 144) in the placebo group. However, admission parasitemias were significantly higher in the NAC treated patients (geometric mean 118,843 parasites/ μ L (95% CI 77,535 to 169,005), compared to 57,178 parasites/ μ L (95% CI 33,327 to 95,531) in the control group ($p=0.021$), and admission (log) parasitemia correlated with (log) parasite clearance time ($r=0.67$, $p<0.0005$). In the Cox regression analysis, adjusting for [log] parasitemia and bilirubin levels at admission, treatment allocation (NAC or placebo) did not significantly affect parasite clearance time (HR 1.17, 95% CI 0.74 to 1.86, $p=0.51$).

Other parasite clearance measures also did not show any difference between treatment groups: mean (95% CI) parasite reduction ratios (PRR) at 24 hours was 11.8 (6.36 to 22.05) in the NAC group and 9.2 (5.57 to 15.25) in the placebo group ($p=0.52$); PRR at 48 hours was 13.3 (3.38 to 52.55) and 38.4 (17.02 to 86.56, $p=0.15$), respectively; the geometric mean time (95% CI) to 50% clearance of parasites (PC50) was 8.1 hours (6.31 to 10.42) in the NAC group and 6.6 hours (5.13 to 8.38) in the placebo group ($p=0.23$); for PC90, times were 19.1 hrs (15.57 to 23.53) and 18.6 hrs (15.88 to 21.87, $p=0.84$), respectively. Thus, there was no evidence that N-acetylcysteine prolonged parasite clearance by artesunate.

Fever clearance time

There was no difference in fever clearance times between the two treatment groups by Kaplan-Meier analysis (Wilcoxon-Breslow, $p=0.49$). Fever clearance time, censoring the fatal cases at the time of death, was 24 hours for both treatment groups (range 6 to 96 hours for the NAC and 6 to 360 hours in the placebo group) and adjustment for [log] parasitemia

at admission and bilirubin in the Cox model did not change this result (HR 0.81, 95% CI 0.43 to 1.53, $p=0.52$).

Mortality

The overall mortality in this severely ill group of patients was 35% (38 of 108 patients, table 3), with 21 (38%) fatal cases in the NAC group versus 17 (33%) in the placebo group, which was not significantly different by survival analysis (Wilcoxon-Breslow, $p=0.52$). The level of supportive and ICU care was higher in Mae Sot General Hospital in Thailand, and mortality was significantly lower at this study site (9% versus 42%, $p=0.003$). Median time to death was 16 hours in both the NAC and placebo treated groups (range 2 to 204 hours in the NAC group and 3 to 288 in the placebo group). In a multiple logistic regression analysis with mortality as the dependent variable, allocation to NAC or placebo did not contribute to the model in addition to the presenting severity symptoms.

Red cell deformability

Admission red cell deformability at a shear stress of 1.7 Pa (as mean elongation index, 95% CI) was assessed in 87 patients. Red cell deformability was lower in fatal cases: 0.182 (0.169 to 0.194), compared to 0.198 (0.189 to 0.207) in patients who survived ($p=0.03$). There was no significant change in red cell deformability in the first 24 hours after admission in either group: mean change (95% CI) 3.1% (−6.5% to 12.8%) in the NAC treated group ($p=0.50$) and 3.1% (−6.1% to 12.3%) in the placebo group ($p=0.49$).

Measures of oxidative stress

Urine F₂-isoP-M concentrations were assessed in 50 of the patients with severe malaria. For comparison, these were also assessed in 15 local volunteers, 13 patients with uncomplicated falciparum malaria, and 4 patients with *P. vivax* infection. After correction for renal function, geometric mean urine F₂-isoP-M were significantly higher in patients with severe malaria when compared to uncomplicated malaria, *P. vivax* and healthy controls (figure 2); the corrected geometric mean F₂-isoP-M concentrations (95% CI, p of difference with value in severe malaria) were 556 pg/ml cr.cl (392 to 787) for patients with severe malaria; for uncomplicated falciparum malaria 183 pg/ml cr.cl (114 to 293, $p=0.002$); in vivax malaria 139 pg/ml cr.cl (23 to 825, $p=0.02$) and in healthy controls 176 pg/ml cr.cl (89 to 350, $p=0.002$). Within the group of patients with severe malaria, there was no difference ($p=0.44$) in corrected urinary F₂-isoP-M concentrations on admission between survivors and fatal cases: geometric mean (95% CI) 613 pg/ml cr.cl (384 to 979) in survivors ($n=33$) and 460 pg/ml cr.cl (271 to 779) in fatal cases ($n=17$). There was no significant correlation with admission hemoglobin, red cell deformability or lactate concentrations. In 32 severe malaria patients, longitudinal data at 24 hours were available. No change in urinary F₂-isoP-M concentrations (corrected for renal function) were observed in the 15 patients treated with NAC (F₂-isoP-M: mean % change, 95% CI = 2.7%, −8.7 to 14.2, $p=0.62$), nor in the 17 patients treated with placebo (6.3%, −2.7 to 16.9, $p=0.22$).

Adverse events

No adverse events related to NAC were observed, including allergic reactions. There was no difference in the proportion of patients presenting with severe complications upon admission between the NAC treatment group and the placebo (table 3).

DISCUSSION

This study evaluated the effects of N-acetylcysteine as adjuvant to intravenous artesunate for the treatment of severe falciparum malaria. A smaller pilot study had shown previously that

clearance of elevated plasma lactate levels and coma recovery times were accelerated by NAC (13). Both parameters have strong prognostic significance in severe malaria. For both variables there was no significant difference between NAC or placebo treated patients in the present study. The main difference with the earlier pilot study was antimalarial treatment with artesunate rather than quinine. Since mortality is lower and parasite clearance times, and possibly lactate clearance times, shorter in patients treated with artesunate compared to quinine, an additional effect of NAC might be more difficult to demonstrate in the current study. Despite strict randomization, patients in the NAC groups had higher levels of parasitemia and bilirubin at admission. However, the results from the Kaplan-Meier analyses were consistent with the results from multivariate analyses, which were controlled for admission parasitemia and bilirubin levels, thereby taking the differences into account.

Mortality, fever clearance time, and complications or adverse events also did not differ between treatment groups. We have recently shown that the antiplasmodial action of artesunate is slightly antagonized by the antioxidant NAC (16). This inhibitory effect was no longer observed when the NAC was added 2 hours after incubation with artesunate. In the current study, NAC was started 2 hours after artesunate administration. Although parasite clearance times were significantly longer in the NAC treatment group in the univariate analysis, when adjusted for admission parasitemia and bilirubin levels, treatment allocation did not have a significant effect on parasite clearance time. Moreover, other measures of parasite clearance, which are less dependent on the admission parasitemia, such as the parasite reduction ratios, were not different between treatment groups. Fever clearance times were also not different between the study groups. This argues against a major antagonistic effect of NAC on the antimalarial activity of artesunate *in-vivo*.

An earlier study in children with severe malaria described an association between an increase in reactive oxygen intermediate production measured by luminol-enhanced chemiluminescence, and severity of disease, especially severe anemia (2). Severe malarial anemia has been linked to oxidative damage of the red cell membrane (9). In our study, urine concentrations of F₂-isoP-M were significantly increased in patients with severe malaria, compared to patients with uncomplicated malaria, vivax malaria, and healthy controls from the same ethnic background. Treatment with an artemisinin, which has oxidative properties, could have contributed to the urinary reactive oxygen intermediates in patients with severe malaria and uncomplicated falciparum malaria, in contrast to those with vivax malaria, who were treated with chloroquine, or healthy controls. We did not observe a correlation with severity of anemia. Recently, it has been shown that plasma L-arginine levels are reduced in severe malaria and that this is associated with decreased nitric oxide (NO) production by nitric oxide synthase (NOS) (26). With low L-arginine levels, NOS generates superoxide rather than NO, which can add to the increase in oxidative stress in severe malaria. Measurement of F₂-isoprostanes metabolites in the urine has emerged as a reliable noninvasive method to determine systemic levels of oxidative stress *in vivo* (27).

Although our study affirms that oxidative stress is increased in severe malaria, intervention with high dose NAC did not show any measurable effect. Labile prostaglandin H₂-like endoperoxides (H₂-isoprostanes) are intermediates in the formation of F₂-isoprostanes. H₂-isoprostanes are reduced to form F₂-isoprostanes, but can undergo rearrangement *in vivo* to form E-ring and D-ring isoprostanes. Since reduction to F₂-isoprostanes is catalyzed by thiols (28), NAC could increase the ratio between F₂-isoprostanes and E2/D2 isoprostanes, obscuring its anti-oxidative effect.

The current study reinforces the prognostic significance of red cell rigidity, as previously shown in both adult and pediatric severe malaria (8,29). Oxidative damage to the red cell membrane has been proposed as a cause for decreased red cell deformability (7), but in our

study deformability did not correlate with urinary F₂-isoprostanes concentrations and was not restored by NAC.

In addition to its anti-oxidative properties (30), additional beneficial effects of NAC have been described in septic shock, such as a vasodilatory effect (10,31-33), associated with a reduction in serum lactate concentrations (34-35), as well as a reduction in nuclear factor kappa-B (36), TNF (34) and IL-8 levels (36-37) with reduced expression of endothelial receptors VCAM-1 and ELAM. However, some of these findings were not reproduced in other studies (38-39). Vasodilatory effects might be less relevant in severe malaria, where microcirculatory hypoperfusion is mainly determined by the mechanical obstruction resulting from sequestration of parasitized red blood cells (8). In patients with severe malaria, no changes in plasma concentrations of TNF α , IL-6, IL-8 or IFN γ could be demonstrated with high dose NAC treatment (13). In the mouse model, antioxidants other than NAC have been shown to reduce mortality (40), and different antioxidant substances vary in their effect on immune function (41). It cannot, therefore, be excluded that other anti-oxidants might have a beneficial effect in human patients. However, the pathogenesis of cerebral malaria in mice is quite distinct from the disease in humans.

In conclusion, this study showed that although measures of oxidative stress were increased in severe malaria, no beneficial effects from the use of high dose NAC as adjunctive treatment to intravenous artesunate could be observed in the setting of the study.

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Figure 1.

Kaplan-Meier curves for lactate clearance times, defined as the time to a plasma lactate concentration below 2 mmol/l (figure 1a, Wilcoxon-Breslow test for equality of survivor functions, $p=0.74$), coma recovery times, defined as the time since admission to a Glasgow Coma Score of 15 (figure 1b, $p=0.46$) and parasite clearance times, defined as the time to a negative peripheral blood slide for asexual parasite forms of *P. falciparum* (figure 1c, $p=0.03^*$). Dotted line represents patients treated with N-acetylcysteine as adjunctive treatment to i.v. artesunate ($n=56$), solid line patients receiving placebo in addition to antimalarial treatment with i.v. artesunate ($n=52$).

Figure 1a. Kaplan Meier curves for lactate clearance time $P=0.74$

Figure 1b. Kaplan Meier curves for coma recovery time $P=0.46$

Figure 1c. Kaplan Meier curves for parasite clearance time* $P=0.03$

*After correction for baseline parasitemia and bilirubin the difference in parasite clearance between treatment group was not significant ($p=0.53$).

Figure 2. Urine F₂-isoprostane metabolites (F₂-isoP-M) concentrations corrected for creatinine clearance in patients with severe falciparum malaria (SM), compared to uncomplicated falciparum malaria (UM), vivax malaria (PV) and healthy local volunteers ('healthy').

Table 1

Baseline characteristics of 108 patients with severe malaria treated with either N-acetylcysteine (NAC) or placebo as adjunctive treatment to i.v. artesunate. F₂-isoP-M is F₂-isoprostane metabolites values are mean (95%CI), unless indicated otherwise.

Variable	NACM (n=56)	Placebo (n=52)	P
Age, yrs	34.2 (30.8 to 37.8)	34.9 (30.9 to 38.8)	0.81
Male /Female patients	44/12	41/11	0.65
Thailand/Bangladesh	13/43	11/41	0.82
Days of fever before admission	5.8 (4.6 to 7.1)	5.6 (4.8 to 6.3)	0.72
Temperature, °C	38.1 (37.8 to 38.4)	38.0 (37.6 to 38.3)	0.64
Systolic blood pressure, mmHg	111 (107 to 116)	112 (106 to 117)	0.96
Oxygen saturation, % *	95 (94 to 96)	96 (95 to 97)	0.12
Glasgow Coma Score **	8 (3 to 15)	9 (3 to 15)	0.61
Hemoglobin, g/dl	10.2 (9.5 to 10.8)	10.1 (9.5 to 10.8)	0.91
Peripheral WBC count, cells/mm ³	9555 (8076 to 11034)	9150 (7624 to 10676)	0.70
Platelet count, cells/mm ³	126026 (102781 to 149272)	139342 (116792 to 161316)	0.40
Parasitemia, /µl *	118843 (77535 to 169005)	57178 (33327 to 95531)	0.02
Serum creatinine level, mg/dl *	1.98 (1.54 to 2.41)	2.04 (1.57 to 2.78)	0.80
Total serum bilirubin level, mg/dl *	5.04 (3.85 to 6.60)	3.41 (2.73 to 4.35)	0.03
Serum albumin, g/100ml	2.91 (2.77 to 3.08)	2.70 (2.53 to 2.85)	0.06
Serum aspartate aminotransferase, U/L *	79 (63 to 100)	80 (62 to 98)	0.91
Serum alanine aminotransferase, U/L *	20 (16 to 25)	23 (17 to 29)	0.46
Serum base excess, mmol/L	-7.9 (-6.7 to -10.0)	-7.1 (-4.9 to -9.3)	0.33
Plasma lactate level, mmol/L *	5.3 (4.6 to 6.7)	4.7 (3.9 to 5.6)	0.36
Red cell deformability, shear stress = 1.7 Pa (EI) (n=87)	0.189 (0.179-0.199)	0.194 (0.183-0.205)	0.53
Urinary F ₂ -isoP-M, pg/ml* (n=50)	627.4 (395.7 to 994.7)	496.9 (288.4 to 856.2)	0.50

* geometric mean (95%CI)

** median (range)

Table 2

Distribution of baseline presenting severity symptoms in patients with severe malaria according to study site and adjuvant treatment group (N-acetylcysteine (NAC) or placebo).

	Thailand (n=23)	Bangladesh (n=85)	NAC (n=56)	Placebo (n=52)	P (NAC vs placebo)
GCS <11	12 (52%)	66 (78%)	42 (75%)	36 (69%)	0.50
Hematocrit < 20% with parasite count > 100,000 /uL	2 (9%)	5 (6%)	3 (5%)	4 (8%)	0.70
Bilirubin > 3.0 mg/dl with parasite count > 100,000 /uL	6 (26%)	27 (32%)	23 (41%)	10 (19%)	0.01
Serum creatinine > 3.0 mg/dl	3 (13%)	17 (20%)	11 (20%)	9 (17%)	0.75
SBP <80 mmHg & cool extremities*	1 (4%)	2 (2%)	2 (4%)	1 (2%)	1
Parasitemia > 10%	7 (30%)	20 (24%)	16 (29%)	11 (21%)	0.37
Venous lactate > 4 mmol/l	10 (43%)	55 (65%)	34 (61%)	31 (60%)	0.90
Venous bicarbonate < 15 mmol/l	3 (13%)	34 (40%)	17 (30%)	20 (38%)	0.37

* SBP = Systolic Blood Pressure

Table 3

Complications according to study site and adjunctive treatment group (N-acetylcysteine (NAC) or placebo) in 108 patients with severe malaria.

	Thailand (n=23)	Bangladesh (n=85)	NAC (n=56)	Placebo (n=52)	<i>P</i> (NAC vs placebo)
Died	2 (9%)	36 (42%)	21 (38%)	17 (33%)	0.60
Mechanical ventilation	3 (13%)	4 (5%)	4 (7%)	3 (6%)	1
Blood transfusion	3 (13%)	8 (9%)	3 (5%)	8 (15%)	0.11
Vasopressor /inotropic drugs	3 (13%)	5 (6%)	5 (9%)	3 (6%)	0.71
Dialysis	2 (9%)	10 (12%)	4 (7%)	8 (15%)	0.22
ARDS	4 (17%)	15 (16%)	9 (16%)	10 (19%)	0.66
Renal failure	2 (9%)	26 (31%)	14 (25%)	14 (27%)	0.82
Black water fever	2 (9%)	12 (14%)	7 (13%)	7 (13%)	0.88
Septicaemia	4 (17%)	17 (20%)	10 (18%)	11 (21%)	0.66
Aspiration pneumonia	3 (13%)	22 (26%)	14 (25%)	11 (21%)	0.63