Clinical Infectious Diseases

MAJOR ARTICLE

The Effect of Regularly Dosed Acetaminophen vs No Acetaminophen on Renal Function in *Plasmodium knowlesi* Malaria (PACKNOW): A Randomized, Controlled Trial

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Background. Acetaminophen inhibits cell-free hemoglobin-induced lipid peroxidation and improves renal function in severe falciparum malaria but has not been evaluated in other infections with prominent hemolysis, including *Plasmodium knowlesi* malaria.

Methods. PACKNOW was an open-label, randomized, controlled trial of acetaminophen (500 mg or 1000 mg every 6 hours for 72 hours) vs no acetaminophen in Malaysian patients aged ≥5 years with knowlesi malaria of any severity. The primary end point was change in creatinine at 72 hours. Secondary end points included longitudinal changes in creatinine in patients with severe malaria or acute kidney injury (AKI), stratified by hemolysis.

Results. During 2016–2018, 396 patients (aged 12–96 years) were randomized to acetaminophen (n = 199) or no acetaminophen (n = 197). Overall, creatinine fell by a mean (standard deviation) 14.9% (18.1) in the acetaminophen arm vs 14.6% (16.0) in the control arm (P = .81). In severe disease, creatinine fell by 31.0% (26.5) in the acetaminophen arm vs 20.4% (21.5) in the control arm (P = .12), and in those with hemolysis by 35.8% (26.7) and 19% (16.6), respectively (P = .07). No difference was seen overall in patients with AKI; however, in those with AKI and hemolysis, creatinine fell by 34.5% (20.7) in the acetaminophen arm vs 25.9% (15.8) in the control arm (P = .041). Mixed-effects modeling demonstrated a benefit of acetaminophen at 72 hours (P = .041) and 1 week (P = .002) in patients with severe malaria and with AKI and hemolysis (P = .027 and P = .002, respectively).

Conclusions. Acetaminophen did not improve creatinine among the entire cohort but may improve renal function in patients with severe knowlesi malaria and in those with AKI and hemolysis.

Clinical Trials Registration. NCT03056391.

Keywords. malaria; *Plasmodium knowlesi*; acute kidney injury (AKI); randomized, controlled trial (RCT); acetaminophen.

The zoonotic parasite *Plasmodium knowlesi* is now the most common cause of malaria in Malaysia [1, 2] and parts of western Indonesia [3, 4]. Acute kidney injury (AKI) is a common complication [5] and a prominent feature of fatal cases [6]. AKI from any cause increases the risk of chronic kidney disease (CKD), cardiovascular events, and mortality [7–9], with risk increasing with severity [10] and duration [11] of AKI. In Ugandan children with severe falciparum malaria, AKI increased the risk of in-hospital and post-discharge mortality and CKD at 1-year follow-up [12].

Hemolysis is thought to be a major contributor to malaria-associated AKI via intravascular release of erythrocytic hemoglobin, followed by oxidation of ferrous to ferric hemoglobin [13, 14]. Further oxidation of ferric to ferryl heme [15, 16] initiates lipid peroxidation and the generation of bioactive molecules, resulting in oxidative stress and injury [17]. Acetaminophen inhibits this process by reducing ferryl heme and preventing the formation of further globin radicals through inhibition of prostaglandin H_2 synthases [18]. Acetaminophen improved renal function in murine models of hemoprotein-induced AKI

Received 25 October 2021; editorial decision 13 February 2022; published online 18 February 2022.

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Clinical Infectious Diseases® 2022;75(8):1379–88

https://doi.org/10.1093/cid/ciac152

[13], in observational studies of adults with sepsis and raised cell-free hemoglobin (CFHb) [19], and in a retrospective study of adults with rhabdomyolysis [20]. A randomized, controlled trial in Bangladeshi adults with severe and moderately severe falciparum malaria demonstrated that regularly dosed acetaminophen improved kidney function, particularly in patients with evidence of hemolysis and oxidative stress [21]. There have been no trials of acetaminophen renoprotection in other hemolytic infections.

We have shown that CFHb is higher in knowlesi malaria than in falciparum malaria and that AKI is at least as common [22]. Therefore, we hypothesized that acetaminophen may be renoprotective in knowlesi malaria.

METHODS

Trial Design and Participants

PACKNOW was a 2-arm, open-label, randomized, controlled trial conducted at 1 tertiary referral hospital and 3 district hospitals in Sabah, Malaysia.

Participants aged ≥5 years hospitalized with microscopy-diagnosed knowlesi malaria were included if they had a temperature >38°C or fever during the preceding 48 hours, were within 18 hours of commencing antimalarial treatment, and provided written informed consent. Patients were excluded if they were pregnant, had a contraindication or allergy to acetaminophen, had known cirrhosis, or drank >6 standard alcoholic drinks per day. Patients without *P. knowlesi* monoinfection confirmation by polymerase chain reaction were retrospectively excluded. The Malaysian Ministry of Health and Menzies School of Health Research (Australia) Ethics Committee approved this study with mutual recognition by the Australian Departments of Defence and Veterans Affairs Human Research Ethics Committee. The study protocol has been published [23].

Randomization, Allocation, and Blinding

Using computer-generated, site-specific block randomization, patients were randomized in a 1:1 ratio to receive either regularly dosed acetaminophen or no acetaminophen, with treatment allocation administered through REDCap electronic software [24]. Laboratory staff were blinded to treatment allocation; however, as placebo tablets were not used, blinding of clinical site investigators or patients was not possible.

Interventions

Acetaminophen (Good Manufacturing Practice [GMP]-produced Paracil, SM Pharmaceuticals, Malaysia) was administered orally every 6 hours for 72 hours by research staff, with patients weighing ≥50 kg receiving 1 g. The protocol suggested that patients who weigh <50 kg would receive 12.5–15 mg/kg of acetaminophen [23]; however, as acetaminophen solution was not readily available, these patients received 500 mg of acetaminophen. In the control arm, acetaminophen was given if temperature remained >39.5°C for >30 minutes despite tepid sponging or if deemed necessary by treating clinicians. All patients received artesunate and/or oral artemether-lumefantrine for malaria at the discretion of treating clinicians according to local guidelines [25].

Study Procedures

Venous blood was collected on enrollment for standard hematology and biochemistry. Peripheral blood parasitemia was assessed by research microscopists on enrollment and every 6 hours until 2 consecutive negative smears were obtained. Serum creatinine was measured on enrollment and then every 12 hours until 72 hours, then at 7 and 28 days. Creatinine was measured in real time at the accredited enrolling hospitals using an automated modified Jaffe alkaline picrate method (Architect c8000 Chemistry Analyzer, Abbot), with the assay traceable to the Isotope dilution mass spectrometry (IDMS) standard. Urine microalbumin was measured on fresh midstream urine (Clinitek 50, Bayer). CFHb was measured at enrollment, at 12 hours, and then daily for 72 hours using enzyme-linked immunosorbent assay (Bethyl Laboratories) on twice-centrifuged citrated plasma. Liver function tests (LFTs) and urine albumin-to-creatinine ratio (ACR) were measured at 0 and 72 hours and at 7 and 28 days. Plasma acetaminophen concentrations were measured using liquid chromatography-mass spectrometry as previously described [26], detailed in the Supplementary Materials.

Outcomes

The primary outcomes were change in log-transformed creatinine from enrollment (hour 0) to 72 hours and change in log-transformed creatinine from enrollment to 72 hours stratified by the presence of hemolysis. Secondary outcomes included longitudinal change in creatinine and the effect of CFHb on longitudinal change in creatinine, including in predefined subgroups of patients with severe knowlesi malaria or AKI; development of AKI at 72 hours; duration of AKI; fever clearance time; parasite clearance time; and safety of acetaminophen in knowlesi malaria, assessed by LFTs, adverse events, and severe adverse events.

Definitions

AKI was categorized using the Kidney Disease: Improving Global Outcomes (KDIGO) criteria [27], with the exception of the urine output criteria as these data were not routinely collected. Baseline creatinine was defined as nadir follow-up creatinine (7–28 days) or, if unavailable, the lowest creatinine during admission or imputed creatinine backcalculated using an inverse modification of diet in renal disease equation [27] assuming a glomerular filtration rate of 100 mL/min/1.73 m^2 (Supplementary Table 1). These definitions of baseline creatinine were chosen according to our published analysis of imputed

baseline creatinine values in acute infection [28]. Patients who met criteria for AKI for ≥7 days after enrollment were considered to have acute kidney disease (AKD) [29]. Severe malaria was defined using modified World Health Organization criteria [30] (Supplementary Figure 1). The a priori plan was to define hemolysis using a receiver operating characteristic curve analysis to identify the CFHb cutoff providing maximal sensitivity and specificity for predicting AKI. However, no relationship was demonstrated between CFHb and creatinine at enrollment, with CFHb peaking at 12 hours post-enrollment (Supplementary Figure 2). We therefore defined hemolysis as peak CFHb greater than the median (≥77 600 ng/mL). A longitudinal acetaminophen exposure variable was used as a proxy for acetaminophen area under the acetaminophen drug concentration-time curve ($AUC_{0–72h}$) in mixed-effects models, defined as the mean measured acetaminophen concentration of each prior time point.

Statistical Methods

A total of 360 patients (180 per arm) was required to give 90% power to detect a 10% difference between arms in log creatinine reduction over 72 hours, with a significance level of 5%, assuming 10% loss to follow-up. Mean and standard deviation (SD) predicted creatinine at enrollment and 72 hours in the control arm were estimated from preliminary data at the study sites [31]. Effect size was estimated based on a clinical trial of acetaminophen in Bangladeshi adults with falciparum malaria [21].

The primary outcome was analyzed using modified intention-to-treat analysis (patient data were excluded if 0-hour or 72-hour creatinine was missing) using analysis of covariance with enrollment creatinine as a covariate. Longitudinal change in creatinine was assessed using area under the creatinine-time curve and mixed-effects models using all available data up to 28 days, with an unstructured covariance matrix and maximum likelihood method of estimation. Longitudinal acetaminophen exposure variable (defined above) was included as a covariate in all mixed-effects models. The model included a separate treatment effect at each post-randomization time point. Variables included in the models were change in creatinine, treatment arm, time, and acetaminophen exposure. Patients who were receiving hemodialysis had a longitudinal creatinine rise of 132.6 μmol/L imputed [32]. Two-group comparisons were conducted using the Student parametric *t* test and the nonparametric Wilcoxon rank sum test. The number of patients who developed AKI was compared using the χ^2 or Fisher exact test. The proportion of patients with ongoing AKI among those with AKI at baseline was compared using Kaplan-Meier survival analysis. Best-fit linear or tobit polynomial regression models were used to estimate the curve of natural logarithm ($\log_e$) parasite counts vs time; the half-life of the curve; and the time to 50%, 90%, 95%, and 99% reduction in parasitemia [33].

RESULTS

Study Population

Between October 2016 and January 2018, 763 patients were assessed for eligibility, with 396 (52%) enrolled. In total, 365 were included in the final modified intention-to-treat primary end point analysis, including 181 in the acetaminophen arm and 184 in the control arm (Figure 1).

Baseline clinical and laboratory data are shown in Table 1. Median age was 36 years (interquartile range [IQR], 25–49; range, 12–96), and 320 (85%) were male. Thirty-three (9%) had severe malaria, 112 (30%) had AKI using the KDIGO criteria, and 5 (1.3%) required hemodialysis. No patients died during the trial. Paracetamol concentrations were higher on enrollment in the control arm compared with the acetaminophen arm (Table 2).

Outcomes

The primary outcome of change in log-transformed creatinine from 0 to 72 hours did not differ between arms, with a reduction of 14.9% (SD, 18.1) and 14.6% (SD, 16.0) in the acetaminophen and control arms, respectively ($P = .81$). In patients with hemolysis (CFHb ≥77 600 ng/mL; n = 182), mean creatinine fell by 17.3% (SD, 19.3) in the acetaminophen arm compared with 14.8% (SD, 15.2) in the control arm ($P = .25$).

Secondary Outcomes

Longitudinal Change in Creatinine

There was no difference between arms in area under the creatinine-time curve at 72 hours, including in the subgroup with hemolysis. Mixed-effects modeling of the change in creatinine over time, including all data up to 28 days, demonstrated a significant interaction between treatment and time at 12 hours post-randomization in the entire cohort ($P = .043$; Figure 2A) but not at any later time points. There was no significant interaction of treatment and time in patients with hemolysis (Figure 2B). Preexisting hypertension or CKD were assessed as potential effect modifiers in the mixed-effect model and did not significantly affect the outcome when included as covariates.

Severe Malaria Subgroup

In the subgroup of 33 patients with severe malaria, the mean proportional reduction in creatinine at 72 hours was 31.0% (SD, 26.5) with acetaminophen compared with 20.4% (SD, 21.5) in the control arm ($P = .12$). In those with severe malaria and hemolysis (n = 22), creatinine fell by 35.8 % (SD, 26.7) over 72 hours in the acetaminophen arm compared with 19.0% (SD, 16.6) in the control arm ($P = .07$; Table 3, Supplementary Figure 3).

Mixed-effects modeling in severe malaria demonstrated a significant interaction of treatment with acetaminophen and time at 48 hours ($P = .004$), 72 hours ($P = .041$), and 1 week

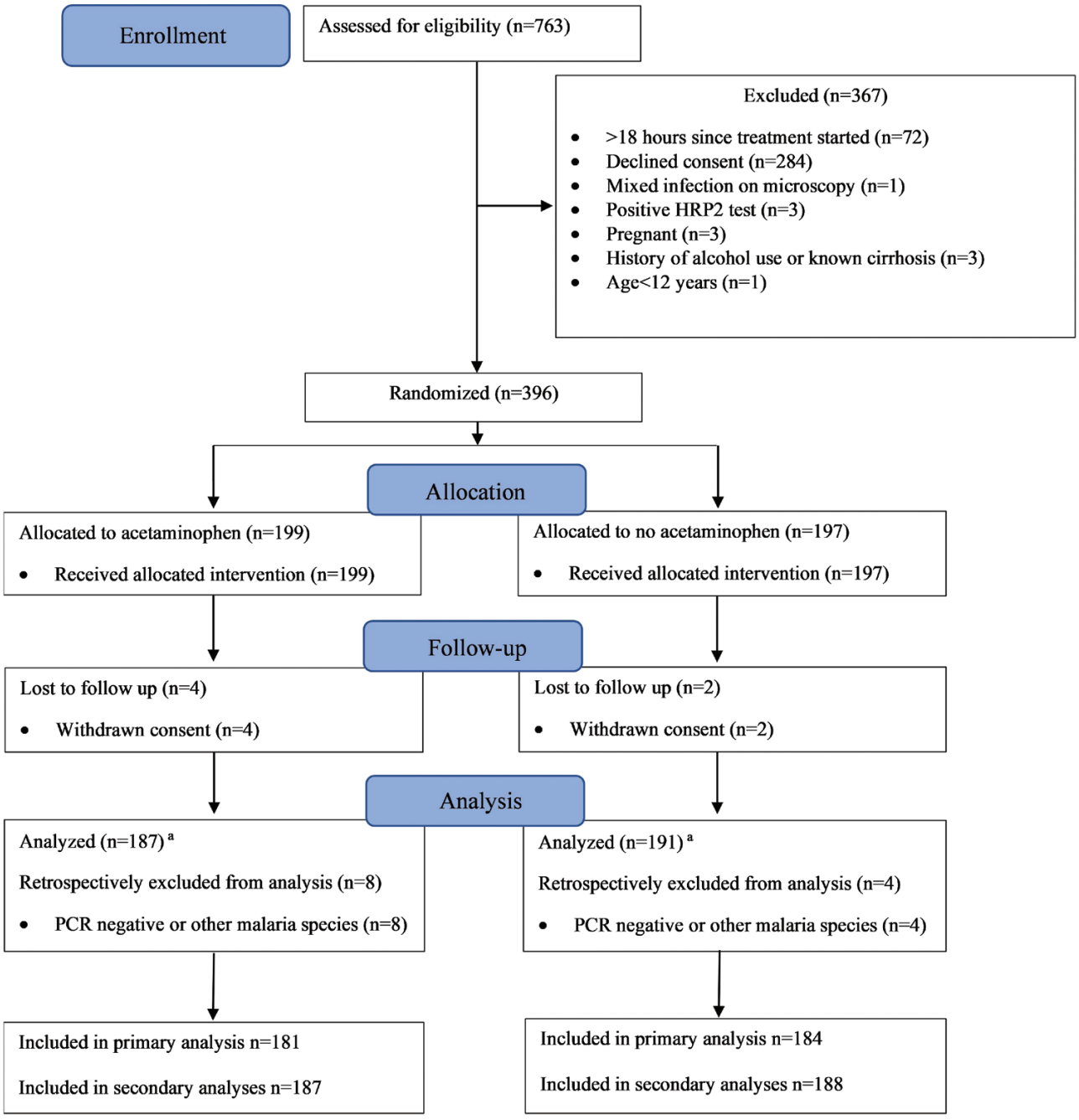

Figure 1. Participant flow diagram. Abbreviations: HRP2, histidine-rich protein 2; PCR, polymerase chain reaction. [a]Thirteen patients did not have either a 0-hour or 72-hour creatinine result and were excluded from the primary analysis (n = 6 in the acetaminophen arm and n = 7 in the control arm). There were 365 patients remaining in the final primary modified intention-to-treat analysis, including 181 patients in the acetaminophen arm and 184 in the control arm.

(P = .002; Figure 2D). No significant interactions of acetaminophen treatment and time were seen in those with severe malaria without hemolysis (Figure 2E).

AKI Subgroup

In the subgroup of patients with AKI included in the Modified intention to treat (MITT) analysis (n = 109), creatinine fell by a mean 28.5% at 72 hours (SD, 21.2) in the acetaminophen arm compared with 27.4% (SD, 16.0) in the control arm (P = .26; Table 3). In those with AKI and hemolysis (n = 56), creatinine fell by a mean 34.5% (SD, 20.7) over 72 hours in the acetaminophen arm compared with 25.8% (SD, 15.8) in the control arm (P = .041; Supplementary Figure 3D).

Mixed-effects modeling showed a significant interaction of treatment and time at 12 hours in the subgroup of patients with AKI (P = .032) and a significant interaction of treatment and

Table 1. Baseline Demographics, Clinical Features, and Laboratory Characteristics

Characteristic	Acetaminophen (n = 187)	Control (n = 191)
Demographics		
Sex, n (%), male	164 (88)	156 (82)
Age, y	35 (26–50)	36 (24–48)
Chronic kidney disease, n (%)	5 (3)	2 (1)
Hypertension, n (%)	23 (12)	16 (8)
Complications at enrollment[a]		
Coma	0	0
Respiratory distress	2	2
Shock	2	5
Jaundice	5	3
Anemia	1	1
Abnormal bleeding	0	0
Hypoglycemia	0	1
Metabolic acidosis	4	3
World Health Organization–defined severe acute kidney injury [b]	6	4
Hyperparasitemia	2	3
Parasitemia ≥20 000/uL	22	26
Severe malaria, n (%)	16 (9)	17 (9)
Number of severity criteria	1 (range, 1–4)	1 (range, 1–3)
Kidney Disease: Improving Global Outcomes stage on enrollment, n (%)		
0	134 (72)	132 (69)
1	40 (21)	52 (27)
2	8 (4)	5 (3)
3	5 (3)	2 (1)
All stages	53 (28)	59 (31)
Clinical examination		
Temperature, °C	37.8 (37.0–38.5)	38.0 (37.2–39.0)
Systolic blood pressure, mm Hg	110 (101– 119)	110 (102–118)
Respiratory rate, bpm	20 (20–20)	20 (20–20)
Oxygen saturation, %	99 (97–99)	98 (98–99)
Weight, mean (±SD), kg	62.4 (±13)	59.6 (±12)
Laboratory investigations[c]		
Parasite count, parasites/µL	2294 (503–9062)	2560 (642–9721)
Sodium, mmol/L	134 (132–137)	134 (132–137)
Potassium, mmol/L	3.8 (3.5–4)	3.8 (3.46–4.1)
Chloride, mmol/L	102 (100–105)	102 (100–104)
Glucose, mmol/L	6.0 (5.4–6.7)	6.2 (5.5–7.5)
Urea, mmol/L	4.9 (3.8–6.7)	5.3 (3.9–6.4)
Serum creatinine, µmol/L	84 (74–99)	86 (74–100)
Hemoglobin, g/dL	13.8 (12.4–14.7)	13.8 (12.5–15.0)
Cell-free hemoglobin, ng/mL	31 100 (16 700–64 900)	27 600 (14 100–58 000)
Maximum cell-free hemoglobin, ng/mL	93 400 (45 700–211 900)	67 300 (39 700–174 500)
Total bilirubin, µmol/L	20.6 (14.1–30.5)	21.5 (14.9–27.0)
Direct bilirubin, µmol/L	11.4 (6.7–17.4)	11.3 (7.0–16.4)
Aspartate aminotransferase, U/L	31 (22–45)	31 (23–45)
Alanine aminotransferase, U/L	36 (24–56)	33 (23–51)
Bicarbonate, mEq/L	21.8 (19.7–24.9)	23.1 (19.6–25.4)
Base excess, mean (SD), mEq/L	–3.0 (±4.0)	–2.0 (±3.4)
Albuminuria, mg/mmol	4.05 (1.74–9.52)	4.6 (2.1–9.5)

Abbreviation: SD, standard deviation.

[a]According to the World Health Organization (WHO) definition of severe *Plasmodium knowlesi* malaria criteria: coma, Glasgow Coma Scale (GCS) <11; respiratory distress, oxygen saturation <92% with respiratory rate >30 breaths per minute; shock, systolic blood pressure <80 mm Hg with cool peripheries or impaired capillary refill; jaundice, bilirubin >50 µmol/L, with parasitemia >20 000/µL and/or creatinine >132 µmol/L; anemia, hemoglobin <7.0 g/dL; abnormal bleeding, including recurrent or prolonged bleeding (from the nose, gums, or venipuncture sites), hematemesis, or melena; hypoglycemia, blood glucose <2.2 mmol/L; metabolic acidosis, bicarbonate <15 mmol/L of lactate >5 mmol/L; severe acute kidney injury, creatinine >265 µmol/L; hyperparasitemia, parasite count >100 000/µL (or >2% infected red blood cells). Data are median (interquartile range) unless otherwise specified.

[b] According to WHO criteria for severe malaria.

[c]All values are median (interquartile range) unless otherwise specified.

[d]Cell-free hemoglobin >77 600.

Table 2. Measured Acetaminophen Concentrations by Arm

	Treatment Arm	
	Acetaminophen (ng/mL)	Control (ng/mL)
Time, hours		
0	1601 (124–6660)	5122 (379–11051)
6	4235 (2706–5788)	2399 (693–5401)
12	4824 (3263–6807)	750 (188–3036)
18	4434 (2996–6507)	436 (118–1107)
24	4461 (3515–6362)	107 (0–430)
30	4341 (3150–6698)	40 (0–141)
36	4233 (3002–5980)	0 (0–55)
42	3967 (2983–5868)	0 (0–110)
48	3687 (2674–5469)	0 (0–0)
54	3363 (2419–4797)	0 (0–0)
60	3334 (2452–4820)	0 (0–0)
66	3129 (1987–4632)	0 (0–0)
72	2959 (2017–4143)	0 (0–0)

Values are median (interquartile range). Acetaminophen levels were higher in the acetaminophen arm compared with the control arm at all time points ($P < .001$ for all comparisons), except for enrollment when concentrations were higher in the control arm ($P = .014$).

time at 72 hours ($P = .027$) and 1 week ($P = .002$) in those with AKI and hemolysis (Figure 2H).

Development and Duration of AKI/AKD

AKI developed after enrollment in 10 (5%) patients in the acetaminophen arm and 15 (8%) in the control arm ($P = .22$). In patients with hemolysis, 2 of 104 (2%) in the acetaminophen arm developed AKI compared with 6 of 85 (7%) in the control arm ($P = .081$). In those with AKI on enrollment (n = 112), there was no difference between arms in the proportion of those with ongoing AKI at 72 hours or the presence of AKD at 7 or 28 days (Supplementary Figure 4).

Albuminuria

At 7 days, albuminuria (urine ACR >3 mg/mmol) was detected in 19% (23 of 121) of patients in the acetaminophen arm compared with 25% (28 of 111) in the control arm ($P = .163$) and in 18% (16 of 89) and 19% (20 of 106), respectively, at 28 days ($P = .512$). In the subgroup of patients with severe malaria and hemolysis, albuminuria was detected at 28 days in 60% (3 of 5) in the control arm and no patients (0 of 7) in the acetaminophen arm ($P = .045$; Supplementary Table 2).

Fever and Parasite Clearance Time

The median time taken for temperature to fall below 37.5°C and remain there for at least 24 hours was 13.5 hours (IQR, 6.6–20.1) in the acetaminophen arm compared with 18.1 hours (IQR, 11.3–6.6) in the control arm ($P = .002$). There was no difference in median parasite clearance time between arms; however, some of the other parasite clearance parameters were slightly increased in the acetaminophen arm (Table 4).

Safety

Median alanine aminotransferase (ALT) was higher at day 3 in the acetaminophen arm (40 U/L; IQR, 25–60) compared with the control arm (30 U/L; IQR, 21–44; $P = .002$); however, no significant rise was seen in ALT between 0 and 72 hours in either arm (Supplementary Figure 5). At day 7, median ALT was higher in the acetaminophen arm (69 U/L; IQR, 43–111) than in the control arm (43 U/L; IQR, 27–57; $P < .0001$) and had increased in both arms from day 3 ($P < .001$ for both comparisons; Supplementary Figure 5). Increased exposure to acetaminophen correlated with a higher ALT at 7 days (correlation coefficient, 0.30; $P < .0001$). No patient met the criteria for Hy's law of hepatotoxicity post-enrollment [34]. There was no difference between arms in adverse events (Table 5) and no serious adverse events.

DISCUSSION

In this randomized, controlled trial, regularly dosed acetaminophen was not associated with renoprotection in the whole cohort of patients with *P. knowlesi* malaria. However, a renoprotective effect was observed in certain predefined subgroups. At 72 hours, a greater reduction in creatinine was observed in patients with AKI and hemolysis. Mixed-effects modeling also demonstrated that regularly dosed acetaminophen was associated with a greater proportional reduction in creatinine at 72 hours and 1 week in patients with severe malaria, with this effect particularly marked in patients with prominent intravascular hemolysis. Similarly, mixed-effects modeling demonstrated that acetaminophen had a beneficial effect at 72 hours and 1 week in patients with AKI and hemolysis. These results are consistent with those from a previous study that demonstrated that regularly dosed acetaminophen improved renal function in Bangladeshi adults hospitalized with severe and moderately severe falciparum malaria, particularly in those with intravascular hemolysis. Taken together, the findings suggest that extending the use of regularly dosed acetaminophen for renoprotection to patients with uncomplicated malaria is not indicated but that acetaminophen should be considered in knowlesi malaria patients with severe malaria or AKI.

The finding that the renoprotective effects of acetaminophen occurred predominantly in those with intravascular hemolysis supports the hypothesis that acetaminophen inhibits CFHb-mediated oxidative damage, an effect also seen in the previous Bangladeshi study where the beneficial effect of acetaminophen was greatest in patients with elevated oxidative stress [21]. AKI that results from raised CFHb and oxidative damage is not unique to malaria [13, 17, 35], suggesting that regularly dosed acetaminophen may have a beneficial effect on renal function in other conditions with cell-free hemoproteins, including rhabdomyolysis.

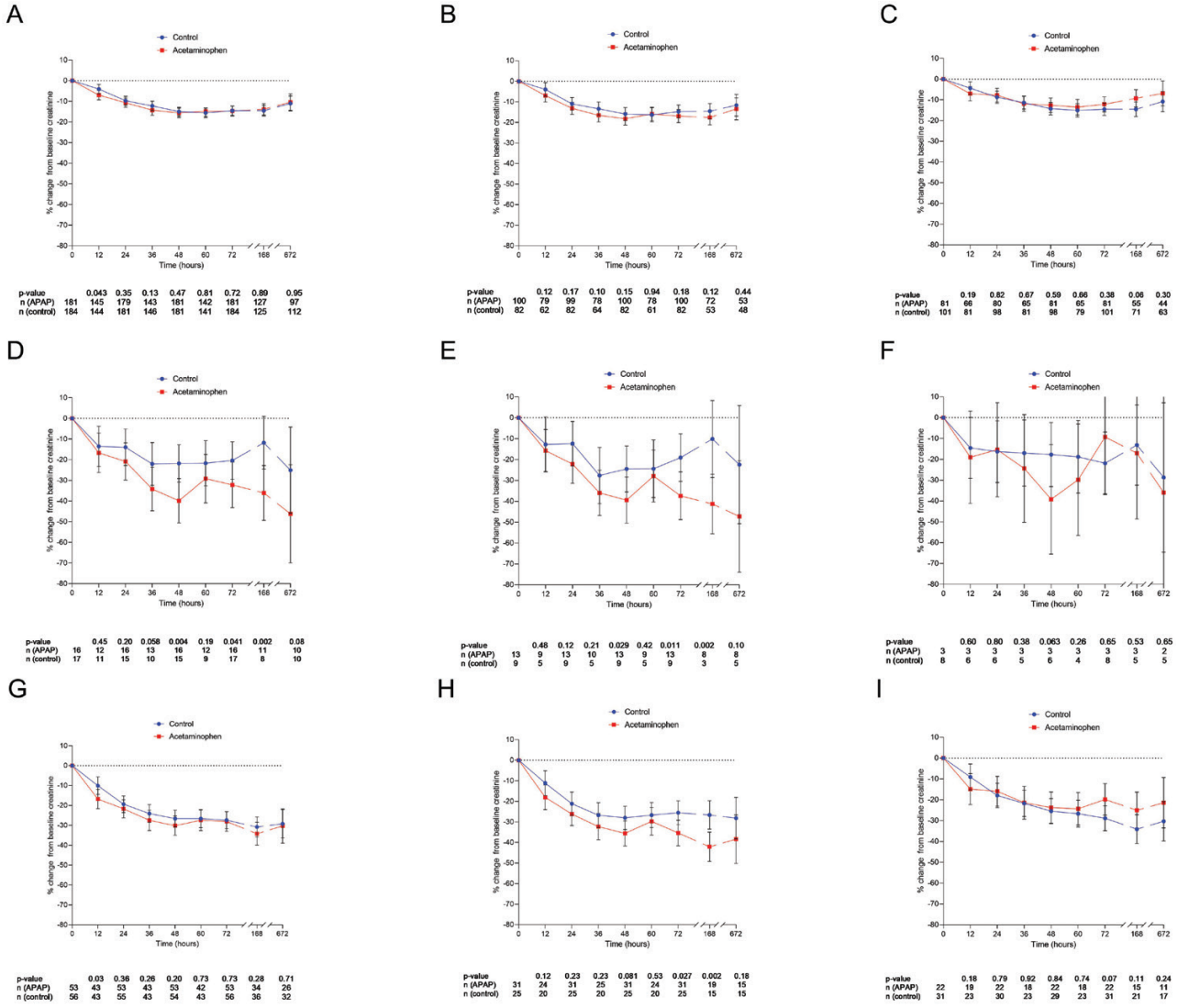

Figure 2. Mixed effects model predicted values of mean percentage change in creatinine from baseline over time from 0 to 672 hours (28 days) in entire cohort (*A*); maximum cell-free hemoglobin (CFHb) ≥77 600 ng/mL (*B*); maximum CFHb <77 600 ng/mL (*C*); severe malaria (*D*); severe malaria and maximum CFHb ≥77 600 ng/mL (*E*); severe malaria and maximum CFHb <77 600 ng/mL (*F*); acute kidney injury (*G*); acute kidney injury and maximum CFHb ≥77 600 ng/mL (*H*); and acute kidney injury and maximum CFHb <77 600 ng/mL (*I*). *P* values below *x*-axes represent fixed effect interaction term of treatment (acetaminophen) × time. Abbreviation: APAP, acetaminophen.

AKI in hospitalized patients increases the risk of adverse clinical outcomes [7–9, 36]. Importantly, even mild, short-term decreases in renal function have adverse long-term consequences [37] that are dependent on severity [10] and duration [11] of AKI. In our study, mixed-effects modeling of change in creatinine over time demonstrated that the effect of acetaminophen was greatest at 7 days in those with severe malaria and those with AKI and hemolysis. Differences in the presence of albuminuria in the severe malaria and hemolysis subgroup were noted at 28 days, suggesting that renoprotective effects of acetaminophen extended beyond the 72-hour treatment period.

A modest increase in some of the parasite clearance parameters was noted in the acetaminophen arm. This association has been previously demonstrated in children with falciparum malaria [38], although was not seen in the Bangladeshi study mentioned above [21], and the clinical relevance is uncertain.

Both ALT and aspartate aminotransferase were higher in the acetaminophen arm compared with the control arm at day 3, and ALT was higher in the acetaminophen arm at day 7. However, ALT rose from day 3 to day 7 in both arms, and no patient met Hy's law of hepatotoxicity [34]. There was no difference in adverse events or serious adverse events between arms, reinforcing the safety of regularly dosed acetaminophen, also demonstrated in falciparum malaria [21].

This study had several limitations. A smaller than expected proportion of patients were enrolled at the tertiary hospital

Table 3. Analysis of Covariance of Change in Log-Transformed Creatinine from Enrollment (Hour 0) to 72 Hours

Group	Total N	% Change in Creatinine [a] (Acetaminophen)	n (Acetaminophen)	% Change in Creatinine [a] (Control)	n (Control)	Analysis of Covariance Coefficient [b]	*P* Value
Entire cohort	365	–14.9 (18.1)	181	–14.6 (16.0)	184	–0.0056 (–0.050 to 0.039)	0.81
Maximum CFHb ≥77 600[c] ng/mL	182	–17.3 (19.3)	100	–14.8 (15.2)	82	–0.040 (–0.11 to 0.028)	0.25
Maximum CFHb <77 600 ng/mL	183	–15.3 (16.2)	82	–14.6 (16.7)	101	–0.038 (–0.021 to 0.097)	0.20
Severe malaria subgroup							
Severe malaria	33	–31.0 (26.5)	16	–20.4 (21.5)	17	–0.20 (–0.45 to 0.53)	0.12
Maximum CFHb ≥77 600 ng/mL	22	–35.8 (26.7)	13	–19.0 (16.6)	9	–0.30 (–0.63 to 0.03)	0.07
Maximum CFHb <77 600 ng/mL	3	–10.2 (13.8)	8	–21.9 (27.1)	3	–0.44 (–0.21 to 1.09)	0.15
Uncomplicated malaria	332	–13.3 (16.4)	165	–14.1 (15.2)	167	–0.006 (–0.037 to 0.025)	0.69
Maximum CFHb ≥77 600 ng/mL	160	–14.5 (16.5)	87	–14.3 (15.1)	73	–0.021 (–0.061 to 0.019)	0.31
Maximum CFHb <77 600 ng/mL	172	–12.0 (16.3)	79	–14.0 (15.5)	93	0.015 (–0.032 to 0.061)	0.54
AKI subgroup							
AKI	109	–28.5 (21.2)	53	–27.4 (16)	56	–0.057 (–0.16 to 0.043)	0.26
Maximum CFHb ≥77 600 ng/mL	56	–34.5 (20.7)	31	–25.9 (15.8)	25	–0.15 (–0.30 to –0.0065)	0.041
Maximum CFHb <77 600 ng/mL	53	–20.0 (19.2)	22	–28.5 (–16.6)	31	0.080 (–0.55 to 0.22)	0.24
No AKI	256	–9.3 (13.2)	128	–9.1 (12.3)	128	–0.0085 (–0.040 to 0.23)	0.60
Maximum CFHb ≥77 600 ng/mL	126	–9.6 (12.6)	69	–9.9 (12.1)	57	–0.0089 (–0.051 to 0.034)	0.68
Maximum CFHb <77 600 ng/mL	129	–9.0 (14.0)	59	–8.5 (12.6)	70	–0.0031 (–0.051 to 0.045)	0.90

Abbreviations: AKI, acute kidney injury; CFHb, cell-free hemoglobin.

[a] Mean percent change in creatinine from 0 to 72 hours (SD).

[b] Analysis of covariance in change in log-transformed creatinine from 0 to 72 hours using baseline creatinine as a covariate. Coefficient (95% confidence interval).

[c] Median maximum CFHb.

site, hence, we had a smaller than expected proportion of patients with severe knowlesi malaria. Given that renoprotective effects of acetaminophen were likely to occur predominantly in this subgroup, the study was underpowered to meet the primary end point. For similar reasons, the majority of patients with AKI were classified as KDIGO stage 1, with only 20 patients (5%) classified as KDIGO stage 2 or 3. With greater hemolysis seen in knowlesi malaria than in falciparum malaria [22], we designed our study to include all patients who presented with knowlesi malaria. While not beneficial in uncomplicated knowlesi malaria, it was nonetheless important to identify those patients unlikely to receive renoprotective benefit from regularly dosed acetaminophen in order to reduce overtreatment of large numbers of patients. The prevalence

Table 4. Parasite Clearance Metrics

	Treatment Arm		
Variable	Acetaminophen (n = 105)	Control (n = 110)	*P* Value
PCT, median (IQR), hours	26.0 (21.3 to 30.9)	25.1 (20.8 to 29.7)	.19
Clearance rate constant, median (IQR), hours	0.27 (0.23 to 0.33)	0.30 (0.25 to 0.35)	.025
Slope half-life, median (IQR), hours	2.61 (2.12 to 3.0)	2.30 (1.97 to 2.73)	.025
PC50, median (IQR), hours	5.7 (3.7 to 9.1)	4.8 (3.6 to 7.4)	.15
PC90, median (IQR), hours	11.8 (9.4 to 15.2)	11.4 (9.0 to 13.7)	.044
PC95, median (IQR), hours	14.6 (11.7 to 18.0)	13.9 (10.9 to 16.5)	.027
PC99, median (IQR), hours	20.5 (17.4 to 24.4)	19.8 (15.5 to 22.3)	.028

PCT: time in hours until first of 2 consecutive negative blood films. PC50: estimated time in hours for parasitemia to reduce by 50% of initial value. PC90: estimated time in hours for parasitemia to reduce by 90% of initial value. PC95: estimated time in hours for parasitemia to reduce by 95% of initial value. PC99: estimated time in hours for parasitemia to reduce by 99% of initial value.

Abbreviations: IQR, interquartile range; PCT, parasite clearance time.

Table 5. Adverse Events and Serious Adverse Events by Arm

	Patients, n (%)		
Adverse Event	**Acetaminophen Arm**	**Control Arm**	***P* Value**
Fever	6 (3)	10 (5)	.18
Rigors	5 (3)	3 (2)	.51
Neurological			
Headache	12 (7)	8 (4)	.36
Dizziness	14 (8)	10 (5)	.80
Retro-orbital pain	2 (1)	1 (0.5)	.60
Confusion	5 (3)	2 (1)	.27
Gastrointestinal			
Nausea	7 (4)	6 (3)	.87
Vomiting	6 (3)	3 (2)	.33
Loss of appetite	17 (9)	16 (9)	.96
Abdominal pain	6 (3)	7 (4)	.64
Diarrhea	3 (2)	1 (0.5)	.34
Skin, rash or itch	7 (4)	2 (1)	.09
Respiratory			
Cough	3 (2)	6 (3)	.33
Difficulty breathing	1 (0.5)	4 (2)	.19
Joint pain	10 (6)	8 (4)	.70
Muscle ache	10(6)	8 (4)	.70
Spontaneous bleeding	2 (1)	0 (0)	.17
Bruising	3 (2)	1 (0.5)	.34

of missing data at enrollment and 72 hours was minimal and similar between arms; however, lower proportions of patients attended follow-up appointments. Importantly, this was not due to death in either arm (and unrelated to any measured outcome). Finally, acetaminophen concentrations on enrollment were higher in the control arm vs the acetaminophen arm, potentially diluting any observed benefit of acetaminophen.

In the largest clinical trial to date in knowlesi malaria, we did not demonstrate a renoprotective effect of acetaminophen in the entire cohort. However, we did find that regularly dosed acetaminophen was safe and had a renoprotective effect in patients with severe malaria and in those with AKI and hemolysis. These findings support increasing evidence that regularly dosed acetaminophen has a renoprotective effect in severe malaria and malaria with AKI and should be considered for use as a cost-effective and readily available adjunctive therapy in these groups, irrespective of causative *Plasmodium* species. It adds to a growing body of evidence that acetaminophen should be investigated as a renoprotective adjunct in other hemolytic disease states.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors thank the participants in this study; clinical and laboratory research staff, including Sitti Saimah binti Sakam, Ema Istiana binti Israk, Noorazela binti Mohamed Yassin, Lizawati binti Pasalam, Jusia binti Raymond, and Maslianah binti Sintom; the hospital directors at the study sites; the medical and nursing staff at Queen Elizabeth Hospital, Kota Kinabalu, Keningau District Hospital, Ranau District Hospital, and Kota Marudu District Hospital for referring patients and facilitating study logistics; and the director-general of the Ministry of Health, Malaysia, for permission to publish this article.

Disclaimer. The views expressed here are those of the authors and do not necessarily reflect the official policy or position of the Australian Defence Force, Joint Health Command, or any extant Australian Defence Force policy.

Financial support. This work was supported by the Australian National Health and Medical Research Council (grants 1037304, 1132975, and 1045156 and fellowships to N. M. A., 1042072, 1135820; B. E. B., 1088738; and M. J. G 1138860); Improving Health Outcomes in the Tropical North: A Multidisciplinary Collaboration HOT NORTH (grant 1131932); and the Australian Centre of Research Excellence in Malaria Elimination. D. J. C. is supported by an Australian Government Prestigious International Research Tuition Scholarship and University Postgraduate Research Scholarship. K. P. was supported by the Infectious Diseases Society of America Education and Research Foundation and National Foundation for Infectious Diseases Young Investigator Merle A. Sande/Pfizer Fellowship in International Infectious Diseases and the Clinician Investigator Program at the University of British Columbia, Canada. A. M. D. is supported by the Wellcome Trust of Great Britain. M. D. E. and G. W. B. report that the Menzies School of Health Research provided consumables for the Liquid chromatography-mass spectrometry (LCMS) measurement of plasma paracetamol concentrations.

Potential conflicts of interest. A. M. D. reports consulting fees paid to MORU by the Novartis Malaria Advisory Council; travel support provided by Gordon Malaria Conference organizers; is a member of the World Health Organization (WHO) Guidelines Development Group for antimalarial treatment, Scientific Advisory Board of the World-Wide Antimalarial Resistance Network, and Scientific Advisory Board of International Severe Acute Respiratory and Emerging Infection Consortium; and is the chair of the Artemisinin-resistance Initiative of the Global Fund Regional Steering Committee. G. S. R. reports grants from the National Institutes of Health's National Institute of Allergy and Infectious Diseases (award 1R01AI160457-01) and the Malaysian Ministry of Health (award NMRR-19-4109-52172) and reports a leadership or fiduciary role on the Infectious Disease Society of Kota Kinabalu. J. T. reports consulting fees paid to Mahidol Oxford Research Unit (MORU) by the Novartis Malaria Advisory Council, is a member of the WHO working group on weight-band dosing harmonization to support the WHO-initiated Global Accelerator for Paediatric Formulations, is chair of the Coronavirus Disease 2019 Clinical Research Coalition, Clinical Pharmacology Working Group, is a member of the American Society for Clinical Pharmacology and Therapeutics (ASCPT) Infectious Diseases steering committee, and is a scientific advisor on the Drugs for Neglected Diseases initiative (DNDi) Programme: 21st Century Treatments for Sustainable Elimination of Leishmaniasis. T. W. Y. reports an international travel grant provided by the Pharmacometrics Japan Conference 2020. All remaining authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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