Age-Related Clinical Spectrum of Plasmodium knowlesi Malaria and Predictors of Severity

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Background. Plasmodium knowlesi is increasingly reported in Southeast Asia, but prospective studies of its clinical spectrum in children and comparison with autochthonous human-only Plasmodium species are lacking.

Methods. Over 3.5 years, we prospectively assessed patients of any age with molecularly-confirmed Plasmodium monoinfection presenting to 3 district hospitals in Sabah, Malaysia.

Results. Of 481 knowlesi, 172 vivax, and 96 falciparum malaria cases enrolled, 44 (9%), 71 (41%), and 31 (32%) children aged ≤12 years. Median parasitemia was lower in knowlesi malaria (2480/μL [interquartile range, 538–8481/μL]) than in falciparum malaria (9600/μL; P < .001) and vivax malaria. In P. knowlesi, World Health Organization–defined anemia was present in 82% (95% confidence interval [CI], 67%–92%) of children vs 36% (95% CI, 31%–41%) of adults. Severe knowlesi malaria occurred in 6.4% (95% CI, 3.9%–8.3%) of adults but not in children; in the commonest severity criterion was acute kidney injury. No patient had coma. Age, parasitemia, schizont proportion, abdominal pain, and dyspnea were independently associated with severe knowlesi malaria, with parasitemia >15 000/μL the best predictor (adjusted odds ratio, 16.1; negative predictive value, 98.5%; P < .001). Two knowlesi-related adult deaths occurred (fatality rate: 4.2/1000 adults).

Conclusions. Age distribution and parasitemia differed markedly in knowlesi malaria compared to human-only species, with both uncomplicated and severe disease occurring at low parasitemia. Severe knowlesi malaria occurred only in adults; however, anemia was more common in children despite lower parasitemia. Parasitemia independently predicted knowlesi disease severity: Intravenous artesunate is warranted initially for those with parasitemia >15 000/μL.

Keywords. Plasmodium knowlesi; malaria; district; clinical epidemiology; children.

Since the initial description of a large focus of zoonotic Plasmodium knowlesi human cases in Sarawak, Malaysia, in 2004 [1], knowlesi malaria has been reported from countries across Southeast Asia [2, 3]. In Malaysia, P. knowlesi now accounts for >90% of all government-notified malaria cases [4–8], with >9500 reported cases from 2012 to 2016 [4, 5]. Plasmodium knowlesi is also increasingly reported in areas of western Indonesia [9, 10]. Difficulties with microscopic diagnosis [2, 11] have limited accurate reporting of the true incidence of knowlesi malaria, with the disease burden likely underestimated [2, 5–7, 12]. Despite great progress in reducing human-only malaria species in many countries [4, 5], increasing numbers of P. knowlesi cases in Southeast Asia threaten regional malaria elimination. Conventional public health measures are unable to target zoonotic transmission to humans from the P. knowlesi reservoir in monkey hosts, particularly outdoors in agricultural or forest areas [13–16].

Prospective studies have described the clinical spectrum of naturally acquired adult knowlesi malaria [17, 18]. Severe knowlesi malaria has been reported in adults in Southeast Asia and in adult travelers returning from these regions [2, 12, 18, 19], with the risk of severe disease at least as high as from Plasmodium falciparum [18]. Deaths from knowlesi malaria have been more common in older adults and have been associated primarily with respiratory distress, hypotension, and acute kidney injury (AKI) [6, 12, 19–21].

Malaria notification data in knowlesi-endemic areas show a median age of 31 years, much higher than that seen with P. falciparum and Plasmodium vivax [7], although 6% (79/1325) of all notified knowlesi malaria cases in Sabah in 2014 occurred in children aged <15 years [6]. With the marked reduction in cases of falciparum and vivax malaria, P. knowlesi now accounts for around 49% of all reported pediatric malaria cases in Sabah [6]. Despite this, there are limited descriptions of knowlesi malaria...
in children [2, 22], or comparisons between zoonotic knowlesi malaria and locally acquired malaria from the human-only species *P. falciparum* and *P. vivax* in district settings.

In this study, we compared the predefined clinical spectrum between children and adults with malaria due to *P. knowlesi* or other *Plasmodium* species infection, and evaluated predictors of disease severity in a coendemic primary care setting.

**METHODS**

**Study Sites and Referral System**

This study was conducted in Kudat Division, northwest Sabah, Malaysia, covering an area of 4623 km² and with a total growth-rate adjusted Malaysian census–estimated population in 2016 of 199,600 people. Each of the 3 districts in this division has a central referral hospital and subdistrict health clinics, consistent with other districts in Sabah. Malaysian Ministry of Health guidelines stipulate that all patients with fever receive microscopic blood slide screening for malaria parasites, with mandatory hospital admission, free treatment, and notification of positive cases [23].

**Subjects**

Patients of all ages presenting to study hospitals with microscopy-diagnosed malaria were enrolled following written informed consent. Children were predefined as age ≤12 years, consistent with Malaysian Ministry of Health pediatric ward admission. Patients were not included in the final analysis if they were pregnant or had *Plasmodium malariae* infection on polymerase chain reaction (PCR), if *Plasmodium* species PCR was not confirmed, or if cross-check research microscopy was negative. A subset of patients with uncomplicated *P. knowlesi* and *P. vivax* malaria was also enrolled in previously reported randomized controlled treatment trials [23–25].

**Study Procedures**

Baseline and longitudinal clinical, laboratory, and epidemiological data were entered using standardized case record forms. Venous blood was taken for baseline investigations and then at the follow-up visit 28 days after treatment initiation. Severe malaria was defined using World Health Organization (WHO) 2014 research criteria [26], including for *P. knowlesi*: hyperparasitemia threshold of 100,000/μL, and jaundice defined as bilirubin >50 μmol/L with parasite count >20,000/μL and/or creatinine >132 μmol/L [18]. Nonsevere anemia was defined using WHO age- and sex-based hemoglobin criteria [27]. AKI was evaluated using Kidney Disease Outcomes Quality Working Group (KDIGO) criteria [28]. Chronic disease was defined as hypertension; diabetes mellitus; ischemic heart disease; hyperlipidemia; or chronic kidney, liver, or respiratory disease.

**Laboratory Procedures**

Microscopic asexual parasite and gametocyte counts were calculated by research microscopists using thick blood smears and quantitated leukocyte count. Standard hospital automated hematology, biochemistry, and microbiology laboratory results were used. Final *Plasmodium* species confirmation was done using PCR [29, 30].

**Statistical Analysis**

We compared between-group differences with analysis of variance or Kruskal-Wallis testing for continuous variables, and Student t test or the Wilcoxon–Mann-Whitney test for 2-group comparisons according to distribution. For categorical variables, χ² or Fisher’s exact test was used. Logistic regression models were fitted to determine a priori predictors of severe malaria based on standard clinical and laboratory WHO 2014 research criteria [26] evaluable at time of acute patient presentation to district hospital settings, including testing for model interactions and collinearity. Receiver operating characteristic (ROC) analysis was used to assess their sensitivity and specificity. Multivariate analysis controlled for age and log parasite density; patients with hyperparasitemia as a sole severity criterion were considered nonsevere.

**Ethical Considerations**

This study was approved by the medical research ethics committees of the Ministry of Health, Malaysia; London School of Hygiene and Tropical Medicine, United Kingdom; and Menzies School of Health Research, Australia.

**RESULTS**

**Demographics**

From October 2012 until April 2016, 811 malaria patients were enrolled (Figure 1). There were 481 *P. knowlesi*, 172 *P. vivax*, and 96 *P. falciparum* malaria cases included in the final analysis. From 2014 to 2015, the estimated minimum yearly malaria incidence in Kudat Division (district hospital presentations with clinical disease) for *P. knowlesi*, *P. vivax*, and *P. falciparum* was 0.79, 0.40, and 0.19 cases per 1000 people per year, respectively. Patients with knowlesi malaria had a median age of 33 years (interquartile range [IQR], 21–49 years), higher than those with vivax (15 years [IQR, 9–30 years]) and falciparum (16 years [IQR, 10–31 years]) malaria (*P* < .001; Figure 2). Patients aged >50 years comprised 107 (22%) of knowlesi malaria cases, compared to 10 (6%) and 14 (15%) for falciparum and vivax malaria, respectively (*P* < .001). A bimodal age distribution was seen for females with both *P. knowlesi* and *P. falciparum* infection. Of *P. knowlesi* cases, 44 (9%) were children, compared to 71 (41%) of *P. vivax* cases and 31 (32%) of those with *P. falciparum* malaria (Table 1; *P* < .001). Only 6 (1.3%) knowlesi cases were <5 years of age, with only 1 infant (<1 year), a 6-week-old with no travel history or forest or plantation exposure. Compared to children
with *P. knowlesi* malaria, adults were more likely to be male (79% vs 57%; *P* = .001), with this relationship also evident for *P. vivax* cases (75% vs 48%; *P* < .001).

**Baseline Features: Children**

Abdominal pain was more common in children with knowlesi compared to vivax malaria (43% vs 13%; odds ratio [OR], 5.2 [95% confidence interval (CI), 2.1–13.1]; *P* < .001), although vomiting occurred more often in those with *P. vivax* (*P* = .033) (Table 1). Children with knowlesi malaria had lower parasite counts than those with *P. vivax* (median, 1722 vs 5967 parasites/μL; *P* < .001) and *P. falciparum* (median, 1722 vs 7392 parasites/μL; *P* < .001). The highest parasite count recorded for a child with knowlesi malaria was 74,365/μL, in an 11-year-old boy with uncomplicated disease. There were 36 (84%) children with knowlesi malaria with nonsevere anemia at presentation, comparable to children with other *Plasmodium* species infection, with no relationship to parasitemia demonstrated. The lowest hemoglobin level of 5.1 g/dL was seen in a 4-year-old child with knowlesi malaria 2 days after treatment, with 2 other children having minimum hemoglobin levels of 7.0 g/dL, all of whom had a parasite count <1000/μL at presentation. Children with knowlesi malaria had lower neutrophil and lymphocyte counts on presentation compared to those with other *Plasmodium* species (*P* = .002). Thirty (68%) children with knowlesi malaria had thrombocytopenia (platelet count <150 × 10^3/μL), including 4 (9%) with a platelet count <50 × 10^3 cells/μL. Frequency of thrombocytopenia in children with knowlesi malaria was comparable to those with *P. vivax* but more common than with *P. falciparum* malaria (OR, 3.0 [95% CI, 1.1–7.7]; *P* = .026). Children with knowlesi malaria were more likely to develop mild to moderate AKI compared to those with *P. vivax* (26% vs 10%; OR, 3.1 [95% CI, 1.1–8.7]; *P* = .030).

**Baseline Features: Adults**

Duration of fever for *P. knowlesi*-infected adults was comparable to both children with *P. knowlesi* and adults with malaria due to...
Table 1. Baseline Demographic, Clinical, and Laboratory Features of Children

| Patient Characteristic | Plasmodium knowlesi | Plasmodium vivax | Plasmodium falciparum | PValue |
|------------------------|---------------------|------------------|-----------------------|--------|
| **Children (age ≤12 y), No. (% total)** | 44 (9.1) | 71 (41.3) | 31 (32.3) | <.001 |
| **Age, y** | 8 (5–10) | 9 (5–10) | 7 (3–10) | .095 |
| **Range** | 0.1–12 | 0.67–12 | 1–12 | |
| **Male sex, No. (%)** | 25 (56.8) | 34 (47.9) | 21 (67.7) | .170 |
| **Previous malaria (self-reported), No. (%)** | 4 (9.1) | 11 (15.5) | 3 (9.7) | .526 |
| **History of chronic disease, No. (%)** | 2 (4.5) | 0 | 0 | .095 |
| **Days of fever** | 5 (3–7) | 5 (3–7) | 4 (3–5) | .751 |
| **Symptoms on enrollment, No. (%)** | | | | |
| **Rigors** | 29 (65.9) | 55 (77.5) | 14 (45.2) | .006 |
| **Headache** | 34 (77.3) | 55 (77.5) | 21 (67.7) | .542 |
| **Vomiting** | 14 (31.8) | 26 (35.2) | 12 (38.7) | .890 |
| **Abdominal pain** | 19 (43.2) | 9 (12.7) | 9 (29.0) | .001 |
| **Diabetes** | 4 (9.1) | 2 (3.6) | 4 (12.9) | .140 |
| **Cough** | 15 (34.1) | 25 (35.2) | 12 (38.7) | .914 |
| **Shortness of breath** | 3 (6.8) | 4 (5.6) | 4 (12.9) | .431 |
| **Myalgia** | 11 (25.0) | 15 (21.1) | 7 (22.6) | .746 |
| **Arthralgia** | 12 (27.3) | 15 (21.1) | 7 (22.6) | .360 |
| **Parasite count/μL** | 1722 (386–4303) | 5967 (1829–13901) | 7392 (1462–36546) | <.001 |
| **Temperature, °C** | 37.1 (36.8–37.9) | 37.4 (36.8–37.8) | 37.1 (36.8–38) | .647 |
| **Fever (≥37.5°C), No. (%)** | 17 (38.6) | 33 (46.5) | 12 (38.7) | .634 |
| **Systolic blood pressure, mm Hg** | 101 (94–109) | 102 (96–110) | 106 (98–112) | .356 |
| **Heart rate, beats/min** | 104 (93–119) | 105 (94–118) | 117 (96–134) | .688 |
| **Respiratory rate, breaths/min** | 24 (22–27) | 24 (22–28) | 26 (24–28) | .081 |
| **Oxygen saturation, %** | 99 (99–100) | 99 (98–100) | 100 (99–100) | .504 |
| **Palpable liver, No. (%)** | 14 (31.8) | 20 (28.2) | 8 (25.8) | .842 |
| **Palpable spleen, No. (%)** | 9 (20.5) | 12 (16.9) | 7 (22.6) | .244 |
| **Parasite count >20000/μL, No. (%)** | 4 (9) | 8 (11) | 12 (39) | .001 |
| **Gametocytes present, no./No. (%)** | 4/35 (11) | 21/66 (32) | 1/11 (9) | .035 |
| **Hemoglobin, g/dL** | 10.6 (9.7–11.3) | 10.1 (9.3–11.2) | 10.3 (9.2–11.6) | .726 |
| **Anemiaa (baseline), No. (%)** | 36 (82) | 56 (79) | 21 (68) | .328 |
| **G6PD deficiency present, no./No. (%)** | 3/6 (2) | 3 (6.8) | 1 (1.5) | .138 |
| **White blood cell count, × 103/μL** | 36–74 (3) | 109–140 (5) | 61–635 (4) | .013 |
| **Neutrophil count, × 103/μL** | 36–74 (3) | 109–140 (5) | 61–635 (4) | .013 |
| **Lymphocyte count, × 103/μL** | 36–74 (3) | 109–140 (5) | 61–635 (4) | .013 |
| **Monocyte count, × 103/μL** | 36–74 (3) | 109–140 (5) | 61–635 (4) | .013 |
| **Platelet count, × 103/μL** | 36–74 (3) | 109–140 (5) | 61–635 (4) | .013 |
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| **Lymphocyte count, × 103/μL** | 36–74 (3) | 109–140 (5) | 61–635 (4) | .013 |
| **Monocyte count, × 103/μL** | 36–74 (3) | 109–140 (5) | 61–635 (4) | .013 |
| **Platelet count, × 103/μL** | 36–74 (3) | 109–140 (5) | 61–635 (4) | .013 |

Data are presented as median (IQR) unless otherwise indicated. Results are from time of enrollment unless otherwise specified.

P values in bold font indicate a value <0.05.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; G6PD, glucose-6-phosphate dehydrogenase; IQR, interquartile range; SD, standard deviation.

aAnemia based on World Health Organization 2011 hemoglobin measurement criteria [27]: age 6–59 months (<100 g/dL), 5–11 years (<115 g/dL), 12–14 years (<120 g/dL), nonpregnant women ≥15 years (<120 g/dL), pregnant women (<110 g/dL), men ≥15 years (<130 g/dL).

*Excluding results positive for skin contaminants.
other *Plasmodium* species (Table 2). Adult *P. knowlesi* cases were less likely to report abdominal pain compared to children with knowlesi malaria (23% vs 43%; OR, 0.40 [95% CI, 0.2–0.8]; *P* = .004; Supplementary Table 1). Adults with knowlesi malaria had lower parasite counts (median, 2541/μL; *P* = .0027) or falciparum (median, 9924/μL; *P* < .001) malaria (Table 2). Age was positively correlated with parasitemia in *P. knowlesi* (r² = 0.15; *P* = .002), but not *P. falciparum* or *P. vivax* infection (Figure 2). Adult *P. knowlesi* cases had a lower risk of anemia at presentation compared to adults with vivax malaria (36% vs 50%, respectively; OR, 0.6 [95% CI, 0.4–0.9]; *P* = .013), and children with knowlesi malaria (36% vs 82%; OR, 0.13 [95% CI, 0.06–0.28]; *P* < .001). As with children, in adult knowlesi malaria parasitemia was not associated with anemia at enrollment after controlling for age. Adults with knowlesi malaria had lower platelet counts than those with other *Plasmodium* species (*P* < .001), with thrombocytopenia more common in adults compared to children with knowlesi malaria (92% vs 68%, respectively; *P* < .001). The risk of AKI was higher in adult *P. knowlesi* patients compared to *P. vivax* (19% vs 10%, respectively; OR, 2.1 [95% CI, 1.1–4.3]; *P* = .033), although this did not remain statistically significant after controlling for age; and was also comparable to that seen in both adults with *P. falciparum* and children with knowlesi malaria. Liver aminotransferases were higher in adults with knowlesi malaria compared to those with *P. vivax* including after controlling for age (*P* = .001). Of the 322 adults with knowlesi malaria who had blood cultures, only 1 grew a noncontaminant isolate, a 14-year-old with *Neisseria meningitidis*.

**Severe Malaria**

*Plasmodium knowlesi* was the most common cause of severe malaria, with 28 of 481 (5.8%) knowlesi cases having severe disease (Table 3), all of whom were adults (28/437; 6.4% [95% CI, 3.9%–8.3%]). Of the severe knowlesi cases, 19 (68%) had severe malaria on presentation and 9 (32%) developed severe complications following commencement of treatment. A single severity criterion defined severe malaria in 16 (57%), with 12 (43%) patients having ≥2 criteria (Table 4). The most common severity criterion was severe AKI (creatinine >265 μmol/L), occurring in 10 (35.7%) severe knowlesi patients, including a single patient who progressed to severe AKI on day 1 of admission. Eight (29%) knowlesi patients had hyperparasitemia >100,000/μL, including 5 (18%) as a sole severity criterion. Parasite counts were higher in severe knowlesi compared to nonsevere knowlesi malaria (median, 42,224 vs 2044 parasites/μL, respectively; *P* < .001). The platelet count was lower in severe vs uncomplicated knowlesi malaria (median, 56 vs 75 × 10⁹ cells/μL, respectively; *P* = .004), neutrophil count was higher (median, 4.9 vs 3.7 × 10⁹ cells/μL, respectively; *P* = .004), and proportion of patients with hyponatremia was higher (48% vs 28%, respectively; *P* = .028). Five (18%) knowlesi patients had documented hypotension, all of whom had other severity criteria including 2 patients with respiratory distress. Empiric antibiotic treatment was given to 9 (32%) patients with severe knowlesi malaria. Of the 4 severe *P. vivax* cases, 2 were children with hyperbilirubinemia and a parasite count >20,000/μL, both with moderate anemia (hemoglobin nadir of 6.8 g/dL and 9.1 g/dL, respectively). The other 2 patients with severe vivax malaria were adults, including a 17-year-old female with severe anemia, and a 53-year-old man with hypotension and respiratory distress. There were 5 patients with severe falciparum malaria (5.2%), including 2 children. No patient with malaria from any *Plasmodium* species had coma.

**Predictors of Severe Malaria**

On multivariate logistic regression controlling for age and parasitemia, independent clinical and parasitological predictors of severe disease in knowlesi malaria included schizont proportion >10%, abdominal pain, and dyspnea (Table 5). Among WHO laboratory severity criteria, creatinine, hemoglobin, bicarbonate, and bilirubin remained independent predictors of severe disease when patients with only a single WHO severity criterion based on these measures were reclassified as nonsevere. A parasite threshold of 15,000/μL had the best-combined sensitivity (74%) and specificity (87%) for predicting severe knowlesi malaria, with an area under the curve of 0.80 (95% CI, 0.71–0.90) and a negative predictive value of 98.5%. Age ≥45 years was the best predictor of hyperparasitemia when controlling for other variables (adjusted OR, 4.9 [95% CI, 1.0–23.9]; *P* = .048). Adults with knowlesi malaria had a higher risk of severe disease compared to adult patients with vivax malaria (OR, 3.4 [95% CI, 0.8–14.5]; *P* = .098), and a comparable risk to falciparum malaria.

**Case Fatalities**

There were 2 deaths attributed to malaria, both *P. knowlesi*, giving an overall *P. knowlesi* case fatality risk of 2 of 481 (0.4% [95% CI, 0.1%–1.5%]), or 2 of 437 (0.5% [95% CI, 0.1%–1.6%]) in adults. The *P. knowlesi*–related deaths were a 62-year-old woman with hyperparasitemia (263,772/μL) and moderate AKI (creatinine: 224 μmol/L), who developed hypotension and acute respiratory distress [6], and a 50-year-old man presenting with severe AKI (creatinine: 609 μmol/L), parasitemia of 71,939/μL, and moderate anemia (hemoglobin: 9.9 g/dL).

**DISCUSSION**

This study is the largest series of *P. knowlesi* malaria cases to date, and the first to prospectively compare the clinical spectrum of disease between adults and children. Although 91% of knowlesi malaria cases were adults, morbidity in children was also demonstrated, with an 11-fold higher risk of anemia at presentation and a similar risk of mild to moderate AKI compared to adults [1, 7]. The majority of adults with knowlesi...
| Patient Characteristic | Plasmodium knowlesi | Plasmodium vivax | Plasmodium falciparum | P Value |
|------------------------|---------------------|-----------------|----------------------|--------|
| Adults (age >12 y), No. (% total) | 437 (90.9) | 101 (58.7) | 65 (67.7) | <.001 |
| Age, y | | | | |
| Median (IQR) | 35 (25–50) | 27 (17–35) | 24 (16–47) | <.001 |
| Range | 13–85 | 13–70 | 1–12 | |
| Male sex, No. (%) | 345 (78.9) | 92 (92.1) | 48 (73.8) | .522 |
| Previous malaria (self-reported), No. (%) | 93 (21.3) | 26 (25.7) | 8 (12.7) | .137 |
| History of chronic disease, No. (%) | 35 (8.0) | 2 (2.0) | 3 (4.6) | .066 |
| Days of fever | 4 (3–7) | 5 (3–7) | 4 (3–6) | .089 |
| Symptoms on enrollment, No. (%) | | | | |
| Rigors | 359 (82.3) | 86 (85.1) | 49 (76.6) | .369 |
| Headache | 389 (89.0) | 93 (92.1) | 56 (86.2) | .369 |
| Vomiting | 105 (24.0) | 26 (24.8) | 18 (27.7) | .734 |
| Abdominal pain | 102 (23.3) | 2 (2.0) | 3 (4.6) | .066 |
| Cough | 153 (35.0) | 32 (31.7) | 7 (10.8) | .726 |
| Shortness of breath | 70 (16.0) | 20 (19.8) | 10 (15.4) | .726 |
| Rash, No. (%) | 19 (4.3) | 3 (3.0) | 1 (1.6) | .92 |
| Examination findings on enrollment | | | | |
| Temperature, °C | 37.4 (37.0–38.1) | 37.4 (36.9–38.0) | 37.0 (36.8–38.1) | <.001 |
| Fever (≥37.5°C), No. (%) | 215 (49.3) | 47 (46.5) | 23 (35.4) | .634 |
| Systolic blood pressure, mm Hg | 120 (110–130) | 115 (106–125) | 112 (106–125) | .004* |
| Heart rate, beats/min | 88 (77–100) | 92 (81–100) | 92 (81–100) | .025a |
| Respiratory rate, breaths/min | 20 (20–24) | 20 (20–22) | 21 (20–22) | .260 |
| Oxygen saturation, % | 98 (97–99) | 99 (98–100) | 99 (98–100) | <.001 |
| Palpable liver, No. (%) | 105 (24.0) | 21 (20.8) | 11 (16.9) | .390 |
| Palpable spleen, No. (%) | 26 (5.9) | 9 (8.8) | 6 (9.2) | .404 |
| Parasite count/μL | 2541 (478–8585) | 3765 (1755–8122) | 9924 (2522–22860) | <.001 |
| Hemoglobin, g/dL | 13.2 (12.1–13.4) | 12.8 (11.2–14.4) | 13.1 (11.3–14.4) | .054 |
| Anemia* (baseline), No. (%) | 156 (36) | 50 (50) | 26 (41) | .041 |
| G6PD deficiency present, no./No. (%) | 4/364 (1.1) | 4/94 (4.3) | 1/48 (2.1) | .117 |
| White blood cell count, × 10³/μL | 20–269 (7) | 53–184 (253) | 33–690 (292) | .013 |
| Neutrophil count, × 10³/μL | 6.1 (5.1–7.4) | 6.5 (5.3–7.8) | 6.5 (5.1–8.0) | .013 |
| Lymphocyte count, × 10³/μL | 3.5 (2.6–4.5) | 4.0 (3.0–5.1) | 4.2 (3.1–5.4) | <.001 |
| Monocyte count, × 10³/μL | 1.4 (1.0–1.9) | 1.4 (1.1–2.0) | 1.4 (1.0–2.3) | .675 |
| Baseline magnesium, μmol/L | 10.7 (9.3) | 10.7 (9.3) | 10.7 (9.3) | .001 |
| Baseline phosphorus, μmol/L | 2.8 (2.3–3.3) | 2.8 (2.3–3.3) | 2.8 (2.3–3.3) | .001 |
| Baseline protein, g/dL | 3.4 (3.2–3.6) | 3.4 (3.2–3.6) | 3.4 (3.2–3.6) | .001 |
| Baseline sodium, mmol/L | 141 (139–143) | 141 (139–143) | 141 (139–143) | .001 |
| Baseline potassium, mmol/L | 4.6 (4.5–4.7) | 4.6 (4.5–4.7) | 4.6 (4.5–4.7) | .001 |

Data are presented as median (IQR) unless otherwise indicated. Includes 2 P. knowlesi and 1 P. falciparum uncomplicated malaria adult patients given single-dose treatment by public health workers prior to enrollment. P values in bold font indicate a value <0.05. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; G6PD, glucose-6-phosphate dehydrogenase; IQR, interquartile range; SD, standard deviation. *Anemia based on World Health Organization 2011 hemoglobin measurement criteria [18]: age 6–59 months (≤100 g/dL), 5–11 years (<115 g/dL), 12–14 years (<120 g/dL), nonpregnant women ≥15 years (<120 g/dL), pregnant women (<110 g/dL), men ≥15 years (<130 g/dL). *Comparisons did not remain statistically significant after controlling for age. **Excluding results positive for skin contaminants; 1 patient with knowlesi malaria was positive for Neisseria meningitidis.
malaria had uncomplicated disease and, compared to those with vivax and falciparum malaria, were older, with a lower risk of nonsevere anemia and a higher risk of thrombocytopenia, consistent with previous reports [1, 17, 18]. The lower parasitemia in both children and adults with clinical illness from \textit{P. knowlesi} infection compared with the human-only \textit{Plasmodium} species may indicate a lower pyrogenic threshold and greater inflammatory response, consistent with poor adaptation of this zoonotic parasite to the human host. Although \textit{P. knowlesi} has a 24-hour blood-stage life cycle in humans, the low parasitemia in most infections may indicate variable efficiency in human red blood cell (RBC) invasion [31]. Only a minority had high parasitemia, with parasitemia an independent predictor of severe knowlesi malaria overall. Notably, there was no coma or convulsions seen in any patient with knowlesi malaria, consistent with previous studies.

### Table 3. Severe Malaria

| Characteristic | \textit{Plasmodium knowlesi} \((N = 481)\) | \textit{Plasmodium vivax} \((N = 172)\) | \textit{Plasmodium falciparum} \((N = 96)\) | \(P\) Value |
|---------------|---------------------------------|------------------------------|---------------------------------|-----------|
| Severe cases  | No. 28                          | 4                            | 5                               | .225      |
|               | \%                                | 5.8                          | 2.3                             |           |
|               | 95\% CI                           | 3.9–8.3                      | 0.6–5.8                         |           |
| Age, y        | Median 53                        | 11                           | 14                              | \.001    |
|               | IQR 43–64                        | 4–35                         | 2–16                            |           |
|               | Range 13–78                      | 3–53                         | 1–26                            |           |
| Child age ≤12 y | No. 0                           | 2                            | 2                               | \.001    |
|               | \%                                | 0                            | 2.8                             |           |
| Male sex      | No. 19                           | 2                            | 4                               | .709     |
|               | \% severe                         | 68                           | 50                              |           |
| Parasitemia/µL| Median 42.225                    | 19.33                        | 297.000                         | \.031    |
|               | IQR 17.221–103.577               | 6.076–43.680                 | 85.505–635.415                 |           |

\(P\) values in bold font indicate a value <0.05.

Abbreviations: CI, confidence interval; IQR, interquartile range.

### Table 4. Severe \textit{Plasmodium knowlesi} Malaria Characteristics

| WHO Severity Criteria                | Definition                                                                 | No. | % Severe \((n = 28)\) | % Total \((n = 481)\) |
|-------------------------------------|---------------------------------------------------------------------------|-----|----------------------|-----------------------|
| Hyperparasitemia                    | Parasite count >100 000/µL                                                | 8   | 28.6                 | 1.7                   |
| Hypotension                         | Systolic blood pressure <70 mm Hg in children or <80 mm Hg in adults      | 5   | 179                  | 1.0                   |
| Impaired consciousness              | Glasgow coma score <11 in adults or Blantyre coma score <3 in children    | 0   | 0                    | 0                     |
| Metabolic acidosis                  | Plasma bicarbonate <15 mmol/L                                             | 3   | 10.7                 | 0.6                   |
| Respiratory distress                | Oxygen saturation <92\% on room air with a respiratory rate >30/min       | 2   | 7.1                  | 0.4                   |
| Jaundice                            | Total bilirubin >50 µmol/L; with parasite count >20 000/µL and/or creatinine level >132 µmol/L | 8   | 28.6                 | 2.0                   |
| Severe acute kidney injury          | Plasma or serum creatinine >265 µmol/L                                   | 10  | 35.7                 | 2.1                   |
| Severe malarial anemia*             | Hemoglobin concentration <5 g/dL in children, and <7 g/dL in adults       | 8   | 28.6                 | 1.7                   |
| Hypoglycemia                        | Blood or plasma glucose <2.2 mmol/L                                      | 1   | 3.6                  | 0.2                   |
| Significant bleeding                | Including recurrent or prolonged bleeding from nose, gums, or venipuncture sites; hematemesis or melena | 1   | 3.6                  | 0.2                   |
| >2 criteria                         |                                                                           | 12  | 42.9                 | 2.5                   |
| Severe criteria developed after presentation |                                                                   | 8   | 28.6                 | 1.7                   |
| Transfer to tertiary hospital       |                                                                           | 15  | 53.6                 | 3.1                   |
| Admitted to ICU                     |                                                                           | 10  | 35.7                 | 2.1                   |
| Death                               |                                                                           | 2   | 7.1                  | 0.4                   |

\(P\) values in bold font indicate a value <0.05.

Abbreviations: ICU, intensive care unit; WHO, World Health Organization.

*Includes 2 \textit{Plasmodium knowlesi} patients with serious underlying medical illness: 1 with worsening of known chronic kidney disease (acute kidney injury) and another with endometriosis-associated bleeding (anemia).
Table 5. Selected Clinical and Laboratory Predictors of Severe Knowlesi Malaria at Presentation

| Variable                          | UM (n = 453) | SM (n = 28) | Univariate Analysis | ROC Analysis | Multivariate Analysis |
|-----------------------------------|--------------|------------|--------------------|--------------|-----------------------|
|                                  | %            | %          | OR (95% CI)        | Sens (%)     | PPV (%)              | aOR (95% CI)          |
| **Age >40 y**                     | 37           | 79         | 6.3 (2.5–16.0)     | <.001        | 79                    | 11.7 (6.3–17.9)       |
| **Age >45 y**                     | 28           | 71         | 6.5 (2.8–15.1)     | <.001        | 71                    | 12.7 (6.3–18.1)       |
| **Age >50 y**                     | 20           | 57         | 5.3 (2.4–11.6)     | <.001        | 57                    | 15.0 (9.5–18.9)       |
| Male sex                          | 23           | 68         | 1.6 (1.7–3.7)      | .245         | 32                    | 8.1 (4.6–14.6)        |
| Previous malaria episode          | 19           | 36         | 2.3 (1.0–5.2)      | .039         | 36                    | 10.3 (4.7–6.6)        |
| Chronic disease                   | 6            | 29         | 5.9 (2.4–14.4)     | <.001        | 29                    | 21.5 (9.2–6.6)        |
| Abdominal pain                    | 24           | 46         | 2.8 (1.3–6.0)      | .010         | 46                    | 10.7 (5.2–21.7)       |
| Shortness of breath               | 14           | 39         | 4.1 (1.8–9.1)      | .001         | 39                    | 15.1 (5.4–47.2)       |
| Parasite count >10 000/μL         | 17           | 79         | 12.6 (4.8–33.0)    | <.001        | 74                    | 21.8 (6.8–87)         |
| Parasite count >15 000/μL         | 12           | 79         | 18.8 (7.1–49.6)    | <.001        | 74                    | 28.6 (5.8–170)        |
| Parasite count >20 000/μL         | 11           | 64         | 11.6 (4.8–28.2)    | <.001        | 61                    | 26.5 (9.7–64.3)       |
| Parasite count >35 000/μL         | 5            | 57         | 18.1 (7.3–45.0)    | <.001        | 52                    | 41.2 (9.3–63.4)       |
| Schizont proportion >1%           | 31           | 57         | 2.9 (1.4–6.4)      | .006         | 57                    | 59.1 (15.3–73)        |
| Schizont proportion >10%          | 8            | 18         | 2.7 (1.0–7.5)      | .060         | 18                    | 19.2 (5.6–63)         |
| Platelets <30 000/μL              | 6            | 18         | 3.7 (1.3–10.5)     | .014         | 18                    | 19.4 (4.9–63)         |
| Platelets <50 000/μL              | 22           | 43         | 2.6 (1.2–5.7)      | .015         | 43                    | 7.0 (1.1–70)          |
| Bilirubin >25 μmol/L              | 21           | 50         | 3.3 (1.8–8.7)      | .018         | 47                    | 9.1 (3.0–29)          |
| Bilirubin >30 μmol/L              | 14           | 46         | 4.1 (1.5–11.3)     | .006         | 41                    | 11.3 (3.1–76)         |
| Bilirubin >50 μmol/L              | 3            | 33         | 10.9 (3.4–35.2)    | <.001        | 29                    | 26.3 (5.3–124)        |
| Creatinine >100 μmol/L            | 24           | 61         | 4.3 (1.9–9.6)      | <.01         | 58                    | 12.3 (3.9–57)         |
| Creatinine >150 μmol/L            | 5            | 28         | 14.9 (6.2–35.7)    | <.01         | 46                    | 9.3 (3.3–29)          |
| Hemoglobin <9 g/dL                | 3            | 43         | 17.8 (6.9–45.6)    | <.01         | 38                    | 40.0 (16.5–77)        |
| Hemoglobin <10 g/dL               | 2            | 46         | 4.6 (1.7–12.3)     | <.03         | 25                    | 16.2 (5.9–50)         |
| Anemia (WHO age criteria)         | 34           | 64         | 4.1 (1.5–11.0)     | .005         | 68                    | 7.6 (1.5–36)          |
| Neutrophil count >4.0/10 000/μL   | 34           | 60         | 2.9 (1.3–6.5)      | .013         | 60                    | 9.0 (3.6–32)          |
| Bicarbonate <20 mmol/L            | 15           | 38         | 3.4 (1.4–8.6)      | .009         | 38                    | 12.7 (5.3–32)         |
| Bicarbonate <18 mmol/L            | 1            | 24         | 22.2 (5.8–84.5)    | <.01         | 24                    | 50.0 (15.2–171)       |
| Age ≥45 y and parasite count >15 000/μL | 5          | 68         | 43.3 (16.4–114.6)  | <.01         | 65                    | 44.1 (17.1–91)        |
| Age ≥45 or parasite count >15 000/μL | 46       | 89         | 10.6 (3.1–36.1)    | <.01         | 87                    | 10.2 (4.6–210)        |
| Age ≥50 or parasite count >20 000/μL | 3         | 50         | 8.4 (3.1–22.1)     | <.01         | 78                    | 11.6 (4.6–183)        |
| Age ≥45 or chronic disease        | 31           | 79         | 8.0 (3.2–20.2)     | <.01         | 79                    | 13.4 (4.8–178)        |

All odds ratios are presented using the complement within the defined variable, for example, age >40 years compared with ≤40 years.

*Values in bold font indicate a value <0.05.

Abbreviations: aOR, adjusted odds ratio; AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; ROC, receiver operating characteristic; Sens, sensitivity; SM, severe malaria; Spec, specificity; UM, uncomplicated malaria; WHO, World Health Organization.

*Univariate analysis: patients with only a single severity criterion (hyperparasitemia, acute kidney injury, anemia, respiratory distress, acidosis, jaundice) were considered nonsevere for the relevant analysis of the corresponding clinical or investigation result.

*Multivariate analysis controlled for: age and ln(parasitemia). Patients with hyperparasitemia as a single severity criterion were considered nonsevere.

*Creatinine, hemoglobin, bicarbonate, and bilirubin at the thresholds shown remained independent predictors of severe disease when patients with only a single WHO severity criterion based on these markers were reclassified as nonsevere.
No child with knowlesi malaria had severe manifestations (although borderline severe anemia was present in one), in contrast to the severe disease found in pediatric falciparum and vivax malaria in this series and elsewhere [26].

A lower proportion of P. knowlesi infections were in children compared to those with P. vivax or P. falciparum. The lower incidence of clinical disease from P. knowlesi infection in infants and also older children has been attributed to epidemiological factors such as lower forest exposure [1, 13, 18], although contributing age-related innate protective mechanisms are plausible [33], and asymptomatic infection has been reported in children [34]. Most children with knowlesi malaria had anemia at enrollment, consistent with a previous retrospective report [22]. Although adults with knowlesi malaria had higher parasite counts, nonsevere anemia was more common in children, suggesting that children may have a higher rate of uninfected RBC destruction and/or greater dyserythropoiesis, although underlying mechanisms and baseline community anemia prevalence require further investigation [35]. Children with knowlesi malaria had lower parasitemia and platelet counts compared to children with either P. vivax or P. falciparum infection, in addition to a lower neutrophil count compared to P. vivax. However, there was a comparable risk of nonsevere anemia and AKI seen in P knowlesi–infected children as in those with P. vivax or P. falciparum.

The proportion of adults with severe disease from P. knowlesi infection was comparable to that seen in P. falciparum. The risk of severe knowlesi malaria in this primary referral setting in Sabah, 6.2% in adults, was similar to district hospital presentations in Sarawak (9.3%) [17], and lower than that demonstrated in a tertiary hospital setting of 29% [18]. Severe AKI was the most frequent severity criterion, and has commonly been reported in other adult studies [17, 18, 32]. Severe anemia was present in a larger proportion of adults with severe knowlesi malaria than in a previous tertiary-referral study, which reported anemia as a severe criterion in only 5% of adults, 1 of whom was spleenectomized [18]. Plasmodium knowlesi parasitemia [18, 32] and age [33] independently predicted severe disease in this study, in addition to abdominal pain and dyspnea, which have not been previously demonstrated. Parasite counts were higher in severe knowlesi malaria than in uncomplicated disease despite no difference in the number of preceding days of fever, which suggests differences in efficacy and tropism of normocyte invasion and parasite multiplication [31]. With age an independent risk factor for both parasitemia and severity, the immunosenescence that occurs with aging [33, 36] may also result in impaired control of parasite multiplication.

The pathophysiological mechanisms in severe knowlesi malaria are not well understood but likely differ from P. falciparum, with coma remaining unreported and a lack of the retinal microcirculatory changes found in severe falciparum malaria [37]. With endothelial activation and systemic inflammation at least as high in response to P. knowlesi as in P. falciparum infection [33, 36], these processes also likely contribute to pathogenesis, particularly with the comparatively low parasite biomass able to produce severe disease observed in this study. The nature and role of microvascular accumulation of parasitized RBCs, a key mechanism of severe knowlesi malaria in rhesus macaques and also observed in a single human autopsy report [21], requires investigation. RBC deformability is reduced in proportion to disease severity in knowlesi malaria [38], however the role of hemolysis and endothelial dysfunction, other key pathogenic mechanisms also present in severe falciparum malaria [39], require further investigation. Phenotypic glucose-6-phosphate dehydrogenase deficiency has been shown to protect against knowlesi malaria [13]. Other host genetic factors related to selection pressure from historical human-only Plasmodium transmission may also modulate disease severity.

Current knowlesi malaria management guidelines in Sabah recommend referral for tertiary care and initial treatment with intravenous artesunate for any patients >50 years of age or with a parasitemia >20,000/μL [18, 40]. Along with appropriate intravenous artesunate administration for severe malaria due to any Plasmodium species, these management guidelines have contributed to a decline in reported malaria case-fatality rate [6, 18, 20]. In the current study, predictors were limited to severe disease given the low case-fatality rate, with a parasite threshold of 15,000 parasites/μL giving a high negative predictive value of 98.5%. A conservative approach would be to recommend early administration of intravenous artesunate initially for any knowlesi malaria case with a parasite count above this threshold, given the potential delay or inability to evaluate other laboratory markers of severe disease in most primary care settings.

In conclusion, although the majority of cases are uncomplicated, P. knowlesi infection causes morbidity at comparatively low parasitemia in both adults and children. Adults are at risk of severe and fatal disease, in contrast to children, among whom this was not demonstrated. A conservative treatment approach utilizing parasite counts to predict severe disease is warranted.

**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**

**Author contributions.** N. M. A., T. W. Y., T. W., B. E. B., and M. J. G. conceived and designed the study; M. J. G., E. S., C. S. W., K. P., and A. C. conducted the study with assistance from G. S. R., T. W. Y., T. W., J. M., B. E. B., C. J. D., and N. M. A; K. P. coordinated the laboratory work; M. J. G. conducted the data analysis, with assistance from N. M. A., T. W. Y., and B. E. B.; M. J. G. wrote the first draft of the manuscript. All authors reviewed the final manuscript.

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