A Prospective Comparative Study of Knowlesi, Falciparum, and Vivax Malaria in Sabah, Malaysia: High Proportion With Severe Disease From *Plasmodium Knowlesi* and *Plasmodium Vivax* But No Mortality With Early Referral and Artesunate Therapy

Bridget E. Barber,[1,4] Timothy William,[1,2,3] Matthew J. Grigg,[1,4] Jayaram Menon,[2,3] Sarah Auburn,[4] Jutta Marfurt,[4] Nicholas M. Anstey,[4,5] and Tsin W. Yeo[4,5]

[1]Infectious Diseases Unit, Department of Medicine, and [2]Clinical Research Centre, Queen Elizabeth Hospital, and [3]Sabah Department of Health, Kota Kinabalu, Sabah, Malaysia; [4]Global and Tropical Health Division, Menzies School of Health Research, Charles Darwin University, and [5]Royal Darwin Hospital, Darwin, Australia

Background. *Plasmodium knowlesi* commonly causes severe malaria in Malaysian Borneo, with high case-fatality rates reported. We compared risk, spectrum, and outcome of severe disease from *P. knowlesi, Plasmodium falciparum*, and *Plasmodium vivax* and outcomes following introduction of protocols for early referral and intravenous artesunate for all severe malaria.

Methods. From September 2010 to October 2011 we prospectively assessed nonpregnant patients aged ≥12 years admitted to Queen Elizabeth Hospital (QEH), Sabah, with polymerase chain reaction–confirmed *Plasmodium* monoinfection. Standardized referral and prereferral intravenous artesunate were instituted at district hospitals.

Results. Severe malaria occurred in 38 of 130 (29%) patients with *P. knowlesi*, 13 of 122 (11%) with *P. falciparum*, and 7 of 43 (16%) with *P. vivax*. The commonest severity criteria in knowlesi malaria included parasitemia >100 000/μL (n = 18), jaundice (n = 20), respiratory distress (n = 14), hypotension (n = 13), and acute kidney injury (n = 9). On multivariate analysis, *P. knowlesi* was associated with a 2.96-fold (95% confidence interval, 1.19–7.38-fold) greater risk of severity than *P. falciparum* ($P = .020$); only parasitemia and schizontemia >10% independently predicted knowlesi severity. Risk of severe knowlesi malaria increased 11-fold with parasitemia >20 000/μL, and 28-fold with parasitemia >100 000/μL. Nearly all (92%) knowlesi malaria patients received oral artemisinin therapy; 36 of 38 (95%) and 39 of 92 (42%) with severe and nonsevere disease, respectively, also received ≥1 dose of intravenous artesunate. No deaths occurred from any species.

Conclusions. *Plasmodium knowlesi* is the commonest cause of severe malaria at QEH, with parasitemia the major risk factor for severity. Early referral and treatment with artesunate was highly effective for severe malaria from all species and associated with zero mortality.

Keywords. malaria; *Plasmodium knowlesi*; Plasmodium vivax.

The simian parasite *Plasmodium knowlesi* is a common cause of human malaria in Malaysian Borneo, accounting for the majority of malaria admissions to several district hospitals throughout Sarawak and Sabah [1–4]. The geographic range of *P. knowlesi* corresponds to the overlapping distribution of the macaque hosts and the

Received 23 July 2012; accepted 9 October 2012; electronically published 19 October 2012.

Correspondence: Nicholas Anstey, PhD, Global Health Division, Menzies School of Health Research, PO Box 41096, Casuarina 0810, Northern Territory, Australia (nicholas.anstey@menzies.edu.au).

Clinical Infectious Diseases 2013;56(3):383–97

DOI: 10.1093/cid/cis902

forest-dwelling *Anopheles leucosphyrus* mosquitoes, extending across Southeast Asia from Indonesia, the Philippines, and southern China to Bangladesh and eastern India, with human infection reported in many of these countries [5].

Plasmodium knowlesi can cause severe and fatal disease [2, 3, 6–8]. The only previous prospective study of knowlesi malaria characterized 10 cases of quinine-treated severe malaria in a district hospital, with 2 deaths [3]. In a retrospective study at Queen Elizabeth Hospital (QEH), a tertiary referral hospital in Sabah, Malaysia, 22 of 56 (39%) patients had severe knowlesi malaria, and 6 (27%) died [7]. In the QEH study, artemisinin-based therapy was associated with faster parasite clearance in uncomplicated and severe knowlesi malaria compared with chloroquine and quinine [7]. As a result, policy changes were instituted in QEH catchment districts for all *Plasmodium* infections, including early tertiary-hospital referral for severe and/or high-parasitemia infections, and prereferral intravenous artesunate for severe malaria. Following these changes, we conducted a prospective study at QEH to compare risk factors, clinical spectrum, and outcome of severe disease from knowlesi, falciparum, and vivax malaria.

METHODS

Study Site and Referral System

QEH, the largest adult hospital in Sabah, serves as the referral center for the West Coast and Kudat divisions, comprising 6 district hospitals (0.6–3 hours' drive from QEH) and a population of 1.14 million. QEH has modern intensive care facilities for invasive ventilation and renal replacement therapy. From 2010, in response to ongoing malaria deaths in Sabah [7, 8], new guidelines were implemented, including tertiary-hospital referral for patients with a thick blood film reported as "4 + " (>10 parasites/high-power microscopy field) or clinical suspicion of severe malaria. Treatment was to commence pretransfer, including intravenous artesunate for all severe malaria, or 1 of 2 nationally available oral artemisinin combination therapies (ACTs; artemether-lumefantrine or artesunate-mefloquine) for nonsevere knowlesi and falciparum malaria; pretreatment blood films accompanied patients. Local health clinics within the Kota Kinabalu area were required to admit all malaria patients to QEH, with treatment commenced on arrival. Patients were hospitalized until blood smears were negative on 2 consecutive days.

Subjects

All patients admitted to QEH with a microscopic diagnosis of malaria from September 2010 to October 2011 were assessed for eligibility. Consecutive nonpregnant patients ≥12 years old were prospectively enrolled in this comparative clinical-pathophysiological study if they were within 18 hours of commencing malaria treatment, had no major comorbidities, and had not previously been enrolled. Patients with mixed-species infection or parasite-negative polymerase chain reaction (PCR) results were retrospectively excluded. Written informed consent was provided by patients or relatives. Approvals were obtained from the Ethics Committees of the Malaysian Ministry of Health and Menzies School of Health Research.

Study Procedures

Standardized data forms recorded epidemiological, clinical, and baseline laboratory (including prereferral) results. Treatment followed hospital guidelines: artemether-lumefantrine for uncomplicated falciparum and knowlesi malaria; chloroquine + primaquine or ACT for uncomplicated vivax malaria;

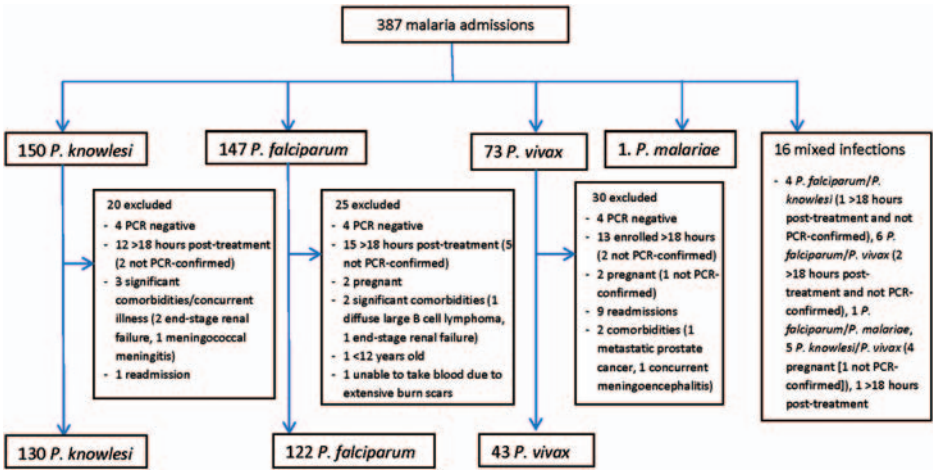

Figure 1. Flowchart showing patient enrollment and exclusions in a prospective comparative study of knowlesi, falciparum, and vivax malaria. Abbreviations: *P.*, *Plasmodium*; PCR, polymerase chain reaction.

Table 1. Baseline Demographic and Clinical Features

Patient Characteristic	*P. knowlesi* (n = 130)	*P. falciparum* (n = 122)	*P. vivax* (n = 43)	*P* Value
Age, years				
Median (IQR)	46 (29–58)	27 (17–40)	24 (18–42)	<.001
Range	14–83	13–78	13–79	
Male sex	96 (74)	87 (71)	33 (77)	.769
Source of referral				<.001
QEH or local health clinic	44 (34)	75 (61)	22 (51)	
District hospital	86 (66)	47 (39)	21 (49)	
Place of residence				<.001
West Coast Division				
Kota Kinabalu, Papar, or Penampang district	33 (25)	73 (60)	20 (47)	
Tuaran, Kota Belud, or Ranau districts	24 (18)	12 (10)	5 (12)	
Kudat Division				
Kudat District	47 (36)	2 (2)	7 (16)	
Pitas District	10 (8)	9 (7)	2 (5)	
Kota Marudu District	9 (7)	23 (19)	9 (21)	
Other	7 (5)	3 (2)	0	
Ethnicity				<.001
Kadazan-Dusun	48 (37)	77 (63)	27 (63)	
Bajau	17 (13)	14 (11)	4 (9)	
Rungus	33 (25)	4 (3)	1 (2)	
Chinese	7 (5)	3 (2)	3 (7)	
Other	25 (19)	24 (20)	8 (19)	
Occupation				.110
Farmer	24 (18)	13 (11)	2 (5)	
Plantation worker	29 (22)	29 (24)	14 (33)	
Other	77 (59)	80 (66)	27 (63)	
Previous malaria (self-reported)	36 (28)	14 (11)	15 (35)	.001
History of chronic disease	31 (24)	12 (10)	3 (7)	.003
Foreign travel past 4 weeks	1 (1)	5 (4)	2 (5)	.114
Use of mosquito nets	59 (45)	46 (38)	16 (37)	.399
Seen a monkey in past 4 weeks	65 (50)	27 (22)	12 (28)	<.001
Forest/plantation exposure				
Live within 20-minute walk of forest	71 (55)	91 (75)	22 (51)	.001
Live within 20-minute walk of plantation	63 (48)	80 (66)	21 (49)	.015
Work within 20-minute walk of forest	64 (49)	59 (48)	13 (30)	.025
Work within 20-minute walk of plantation	57 (44)	55 (45)	18 (42)	.174
>4 hours in forest in past 4 weeks	87 (67)	72 (59)	21 (49)	.091
>4 h in plantation in past 4 weeks	65 (50)	63 (52)	19 (44)	.701
Night in forest in past 4 weeks	69 (53)	55 (45)	15 (35)	.098
Night in plantation in past 4 weeks	44 (34)	39 (32)	8 (19)	.162
Any of the above	119 (92)	111 (91)	34 (79)	.054
Days of fever, median (IQR)	5 (3–7)	5 (3–7)	5 (3–7)	.595
Symptoms				
Rigors	109 (84)	107 (88)	36 (84)	.647
Vomiting	39 (30)	61 (50)	17 (40)	.005
Cough	63 (48)	57 (47)	16 (37)	.432
Shortness of breath	22 (17)	12 (10)	5 (12)	.239
Abdominal pain	40 (31)	40 (33)	10 (23)	.504
Diarrhea	24 (18)	20 (16)	9 (21)	.786
Headache	118 (91)	107 (88)	40 (93)	.546
Myalgias	61 (47)	51 (42)	13 (37)	.483
Arthralgia	75 (58)	62 (51)	18 (42)	.174

Table 1 continued.

Patient Characteristic	*P. knowlesi* (n = 130)	*P. falciparum* (n = 122)	*P. vivax* (n = 43)	*P* Value
Examination findings on enrollment				
Temperature, °C, median (IQR)	37.5 (36.8–38.4)	37.6 (37–38.8)	37.1 (36.7–38.2)	.053
Systolic blood pressure, mm Hg, mean (SD)	118 (19.7)	116 (16.6)	114 (12.4)	.334
Heart rate, beats/min, mean (SD)	90 (15.1)	93 (17.7)	90 (19.2)	.296
Respiratory rate, breaths/min, mean (SD)	27 (6.0)	27 (6.1)	25 (5.4)	.179
Oxygen saturation, %, median (IQR)	97.5 (96–99)	98 (97–99)	98 (97–99)	.013
Palpable liver	52 (40)	32 (26)	13 (30)	.062
Palpable spleen	43 (33)	39 (32)	13 (30)	.939

Data are No. (%), unless otherwise indicated.

Abbreviations: IQR, interquartile range; *P.*, *Plasmodium*; QEH, Queen Elizabeth Hospital; SD, standard deviation.

and intravenous artesunate for severe malaria, or if deemed warranted by the treating clinician. Severe malaria, not previously defined by the World Health Organization (WHO) for nonfalciparum species, was defined as the presence of ≥1 of the following modified 2010 WHO criteria for severe falciparum malaria [9, 10]: unrousable coma (Glasgow Coma Scale score <11); multiple (>2) convulsions; respiratory distress (respiratory rate >30 breaths per minute and oxygen saturation <94%); hypotension (systolic blood pressure ≤80 mm Hg); jaundice (bilirubin >43 μmol/L plus parasitemia >100 000/μL [*Plasmodium falciparum*] or >20 000/μL [*P. knowlesi* and *Plasmodium vivax*] and/or creatinine >132 μmol/L); severe anemia (hemoglobin <7.0 g/dL); significant abnormal bleeding; hypoglycemia (blood glucose <2.2 mmol/L); metabolic acidosis (bicarbonate <15 mmol/L or lactate >5 mmol/L); acute kidney injury (AKI; creatinine >265 μmol/L); hyperparasitemia (parasite count >10% [*P. falciparum*] or >100 000/μL [*P. knowlesi*] [3]).

Patients were assessed daily, including blood films and automated cell counts. Parasite and fever clearance times were defined as the first of 2 consecutive days with a negative blood film and temperature ≤37.5°C, respectively.

Laboratory Procedures

Parasitemia was reported as parasites per 200 leukocytes or 1000 erythrocytes and converted to parasites per microliter. When pretreatment slides were unavailable (6%), referring-hospital microscopy was used and the "1+ to 4+" grade converted into parasites per microliter using relevant median parasite densities. Hematology, biochemistry, blood cultures (where possible), acid-base parameters, and lactate (by bedside blood analysis; iSTAT System) were obtained on admission. Parasite species were identified by PCR, as previously described [11, 12].

Statistical Analysis

Data were analyzed using Stata software, version 10.1. Depending on distribution, analysis of variance or Kruskal-Wallis testing was used to compare intergroup differences for continuous variables, Student *t* test or Mann-Whitney test for post hoc pairwise comparisons, χ^2/Fisher exact test for intergroup differences between categorical variables, and Pearson or Spearman correlation coefficients for associations between parasitemia and other variables. Baseline predictors of severe disease were determined by multiple logistic regression, and linear regression assessed associations between laboratory variables. Receiver operating characteristic (ROC) analysis assessed sensitivity and specificity of parasitemia cutoffs for severe disease.

RESULTS

Baseline Characteristics

In total, 295 patients with PCR-confirmed *Plasmodium* monoinfection were enrolled (Figure 1, Table 1), with *P. knowlesi* the most common species (n = 130; 44%; Table 1). Most (n = 86; 66%) knowlesi malaria patients were referred from district hospitals, most commonly Kudat (n = 39; 30%). In contrast, 61% and 51% of patients with falciparum and vivax malaria, respectively, were referred from QEH emergency department or local primary care clinics (Table 1). Knowlesi malaria patients were older than those with other malaria species (Table 1); this difference remained significant after excluding patients referred from district hospitals (median age, 35, 28, and 23 years for knowlesi, falciparum, and vivax malaria, respectively, after exclusion of referred patients, $P = .005$). Females were older than males among patients with knowlesi (median age, 54 vs 43 years, $P = .011$) and falciparum (median age, 29 vs 25 years, $P = .145$), but not vivax malaria.

Nearly all patients with knowlesi and falciparum malaria reported forest or plantation exposure (Table 1). A greater proportion of *P. falciparum* patients lived in or near forested areas; however, *P. knowlesi* patients were more likely to have

Table 2. Investigation Results

	P. knowlesi (n = 130)			*P. falciparum* (n = 122)			*P. vivax* (n = 43)				
	Nonsevere (n = 92)	Severe (n = 38)	*P* Value	Nonsevere (n = 109)	Severe (n = 13)	*P* Value	Nonsevere (n = 36)	Severe (n = 7)	*P* Value	*P* Value (All Species, Nonsevere)	*P* Value (Severe *P. knowlesi* vs Severe *P. falciparum*)
Parasite count, parasites/μL	4837 (1576–14 641)	80 359 (25 874–168 279)	<.0001	10 500 (4014–32 267)	72 270 (27 905–273 909)	.002	4753 (2369–10 316)	10 243 (4387–40 895)	.310	.0001	.778
Parasite count >100 000/μL, No. (%)[a]	0 (0)	18 (47)	<.0001	3 (2.8)	6 (46)	<.0001	0 (0)	0 (0)	NA	NA	1.000
Hemoglobin, g/dL, mean (SD)	12.8 (1.56)	12.1 (1.82)	.027	13.1 (1.76)	12.2 (3.09)	.157	12.3 (1.88)	13.7 (1.30)	.068	.071	.869
Hemoglobin nadir, g/dL	11.9 (10.7–12.9)	9.35 (7.8–11.2)	<.0001*	11.9 (10.6–12.8)	9.4 (8.3–10.5)	.001*	11.4 (9.75–12.4)	11.2 (10.7–12)	.934	.132	.728
Hemoglobin fall, g/dL	1.05 (0.45–1.55)	2.5 (1.4–3.1)	<.0001*	1.3 (0.7–2.1)	2.7 (1–3.5)	.042*	1 (0.4–1.5)	2.2 (1.4–3.2)	.002*	.020	.974
Day of hemoglobin nadir	2 (1–3)	3 (2–3)	.0004	2 (1–3)	2 (1–4)	.712	2 (1–2)	1 (1–2)	.498	.132	.601
White blood cell count, $\times 10^3$/μL	6.05 (4.95–7.25)	6.60 (5.60–9.80)	.031	5.9 (4.9–7.3)	5.0 (3.7–7.2)	.148	6.15 (4.75–7.35)	6.5 (4–8.2)	.921	.699	.010
Neutrophil count, $\times 10^3$/μL	3.41 (2.48–4.45)	4.70 (3.49–7.57)	.0004*	3.52 (2.59–4.63)	3.02 (2.29–4.52)	.800	3.48 (2.77–4.51)	3.30 (2.25–5.58)	.921	.772	.036
Lymphocyte count, $\times 10^3$/μL	1.55 (1.02–1.98)	1.44 (1.01–1.89)	.728	1.26 (0.96–1.75)	0.70 (0.55–1.45)	.010	1.27 (0.68–1.76)	1.14 (0.38–2.24)	.921	.051	.005*
Monocyte count, $\times 10^3$/μL	0.92 (0.64–1.20)	0.95 (0.68–1.29)	.933	0.82 (0.58–1.06)	0.46 (0.36–0.71)	.007	0.81 (0.45–1.07)	0.39 (0.31–0.59)	.038	.046*	.008
Platelet count, $\times 10^3$/μL	51 (35–81)	29 (20–49)	.0001	71 (40–111)	24 (19–63)	.003	68 (49–106)	29 (18–71)	.029	.004*	.940
Platelet count nadir, $\times 10^3$/μL	42 (25–62)	27 (18–46)	<.0001	59 (37–89)	24 (16–49)	.001	64.5 (45.5–98.5)	29 (18–62)	.026	.004*	.905
Day of platelet count nadir	0 (0–1)	0 (0–1)	.768	0 (0–1)	0 (0–1)	.498	0 (0–1)	0 (0–1)	.292	.082	.906
Platelet count nadir <150 $\times 10^3$/μL, No. (%)	91 (99)	38 (100)	.710	101 (93)	13 (100)	.395	35 (97)	7 (100)	.837	.062	NA
Platelet count nadir <50 $\times 10^3$/μL, No. (%)	46 (50)	32 (84)	<.0001	43 (39)	10 (77)	.011	13 (36)	4 (57)	.265	.213	.414
Platelet count nadir <20 $\times 10^3$/μL, No. (%)	3 (3.3)	11 (29)	<.0001*	4 (3.7)	5 (38)	.001*	0 (0)	2 (29)	.023	.765	.378
Creatinine, μmol/L	92.5 (77.5–110)	141 (101–213)	<.0001	87 (72–102)	106 (80–184)	.033	78.5 (67.5–98.5)	97 (84–143)	.065	.043	.177
Sodium, mmol/L, mean (SD)	134 (3.22)	131 (4.59)	<.0001*	132 (4.30)	130 (5.31)	.063	136 (3.24)	136 (5.27)	.884	<.0001*	.360
Bilirubin, μmol/L	16.6 (12.9–24.8)	42.1 (26–66.8)	<.0001*	19.1 (12.3–29.3)	54.4 (27.1–76.7)	<.001*	17.1 (11.6–23.1)	25.5 (10.2–68)	.147	.427	.517

Table 2 continued.

	P. knowlesi (n = 130)			*P. falciparum* (n = 122)			*P. vivax* (n = 43)				
	Nonsevere (n = 92)	Severe (n = 38)	*P* Value	Nonsevere (n = 109)	Severe (n = 13)	*P* Value	Nonsevere (n = 36)	Severe (n = 7)	*P* Value	*P* Value (All Species, Nonsevere)	*P* Value (Severe *P. knowlesi* vs Severe *P. falciparum*)
Glucose, mmol/L	6.7 (5.8–7.8)	7.7 (6.4–9.5)	.009	6.6 (5.6–7.6)	6.2 (3.9–8.3)	.497	6.2 (5.1–7.2)	6.8 (5.8–7.6)	.222	.108	.048
Albumin, g/dL, mean (SD)	30.5 (4.73)	26.6 (5.32)	.0001*	31.3 (4.91)	26.1 (4.39)	<.001*	31.8 (5.36)	25.43 (4.65)	.006*	.338	.749
AST, IU/L	39 (26–49)	57.5 (44–79)	<.0001*	35 (27–52)	46 (25–67)	.398	31 (24–38)	42 (27–64)	.046*	.027	.370
ALT, IU/L	35 (21–56)	35.5 (19–51)	.624	34 (21–49)	26 (18–60)	.490	22 (16–44)	25 (20.2–60)	.458	.032	.496
Bicarbonate, mmol/L, mean (SD)	24.8 (3.76)	21.6 (4.91)	<.0001*	24.1 (2.73)	18.4 (4.42)	<.0001*	25.47 (3.87)	22.87 (4.25)	.118	.039	.059
Lactate, mmol/L	1.13 (0.86–1.37)	1.52 (1.14–2.30)	.0003	1.22 (0.91–1.62)	1.76 (1.37–2.24)	.014*	1.23 (0.9–1.55)	1.57 (1.24–2.28)	.052	.319	.472
Percentage of trophozoites	49 (7.59–92.1)	42 (1.96–91.8)	.465	0 (0–0)	0 (0–0)	.878	75.2 (23.5–93.8)	56.3 (17.0–74.0)	.327	.0001*	.0001*
Percentage of schizonts	0 (0–1.25)	0.79 (0–11.1)	.042	0 (0–0)	0 (0–0)	.340	0 (0–1.27)	0 (0–1.42)	.674	.0001*	.001*

Unless otherwise indicated, results are of investigations performed at baseline, and data are shown as median (interquartile range). Baseline investigations include those taken from district hospitals and/or Queen Elizabeth Hospital, and are all taken prior to or within 18 hours of start of antimalarial treatment.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not assessed; *P.*, *Plasmodium*; SD, standard deviation.

[a] *P* value for proportion of all *P. knowlesi* patients with parasite count >100 000/μL (14%) versus proportion of all *P. falciparum* patients with parasite count >100 000/μL (7%) = 0.107.

*Remains significant ($P < .05$) after adjusting for age and parasite count, using linear or logistic regression for continuous or categorical variables, respectively.

seen a monkey in the preceding month (Table 1). Among *P. knowlesi* patients, 27 of 57 (47%) aged ≥50 years were farmers or plantation workers compared with 26 of 73 (36%) <50 years ($P = .176$). Median fever duration was 5 days for all malaria species, with minimal differences in clinical characteristics (Table 1).

Laboratory Investigations

Comparing baseline laboratory investigations (Table 2), patients with nonsevere knowlesi malaria had lower parasitemia than those with nonsevere falciparum ($P < .001$), but not vivax malaria ($P = .951$). Moderate anemia (hemoglobin nadir <10 g/dL) was common in all species, occurring in 36 (28%), 24 (20%), and 10 (23%) patients with knowlesi, falciparum, and vivax malaria, respectively ($P = .326$). Thrombocytopenia (platelet count $<150 \times 10^3$ platelets/μL) occurred in all but 1 patient with knowlesi malaria, a 49-year-old splenectomized woman (nadir 260×10^3 platelets/μL). Thrombocytopenia was also absent in another splenectomized patient with nonsevere knowlesi malaria who was excluded (antimalarials for >18 hours), but did occur in a splenectomized patient with severe knowlesi malaria (nadir 27×10^3 platelets/μL). Platelet counts recovered quickly, with nadir occurring by day 1 in all knowlesi patients.

Table 3. Characteristics and Severity Criteria Among Patients With Severe Malaria

	P. knowlesi (n = 38)	*P. falciparum* (n = 13)	*P. vivax* (n = 7)
Age, years			
Median (IQR)*	55 (47–62)	33 (17–45)	43 (15–54)
Range	20–74	13–54	15–54
Severity criteria, No. (%)			
Hyperparasitemia[a]	18 (47)	2 (15)	NA
Respiratory distress	14 (37)	4 (31)	1 (14)
Hypotension	13 (34)	6 (46)	5 (71)
Jaundice[b]	20 (53)	9 (69)	2 (29)
Acute kidney injury	9 (24)	3 (23)	0
Metabolic acidosis	4 (11)	4 (31)	0
Severe anemia	2 (5)	2 (15)	0
Abnormal bleeding	2 (5)	1 (8)	1 (14)
Multiple convulsions	0	0	1 (14)
Hypoglycemia	0	0	0
Coma	0	0	0

No significant ($P < .05$) differences in severity criteria were demonstrated between species, except for hyperparasitemia.

Abbreviations: IQR, interquartile range; *P.*, *Plasmodium*.

[a] Hyperparasitemia was defined as >100 000 parasites/μL for *P. knowlesi* and >10% parasitemia for *P. falciparum*.

[b] With parasite count >20 000 parasites/μL (for *P. knowlesi* and *P. vivax*) or >100 000 parasites/μL (for *P. falciparum*), and/or creatinine level >132 μmol/L.

*$P < .0001$ for *P. knowlesi* versus *P. falciparum*.

Severe Knowlesi Malaria

Thirty-eight (29%) knowlesi malaria patients had severe disease (Tables 3 and 4), compared to 13 (11%) with falciparum malaria ($P < .001$) and 7 (16%) with vivax malaria ($P = .111$) (Table 5). Excluding district referrals, 7 of 44 (16%) and 5 of 75 (7%) patients had severe knowlesi and falciparum malaria, respectively ($P = .124$). Among severe knowlesi patients, 36 (95%) met severity criteria on admission, and 2 (5%) after admission (patients 18 and 24, Table 4). Thirteen patients had 1 severity criterion, 14 had 2 criteria, and 11 had ≥3 criteria. The commonest complications included hyperparasitemia (n = 18, 47%), and jaundice with either renal impairment or parasitemia >20 000 parasites/μL (n = 20, 53%). Only 3 (8%) patients with hyperparasitemia, 1 with creatinine of 173 μmol/L, did not meet other severity criteria. Respiratory distress was common (n = 14, 37%); however, only 2 patients had acute respiratory distress syndrome (ARDS; partial pressure of arterial oxygen:fraction of inspired oxygen < 200 mm Hg) and neither required mechanical ventilation. Hypotension occurred in 13 (34%) patients. Preantibiotic blood cultures, taken in 24 severe cases (including 10 with hypotension), were all negative.

AKI occurred in 9 (24%) patients, with 2 requiring acute hemodialysis but none requiring long-term renal replacement therapy. Severe anemia occurred in 2 patients, both with AKI, and an additional 5 were transfused for hemoglobin 7.0–7.8 g/dL. Two patients had significant abnormal bleeding; 1 with bleeding gastric ulceration and 1 with severe epistaxis. The latter patient, with prior idiopathic thrombocytopenic purpura and platelet count 122×10^3 platelets/L 6 weeks before admission, had a platelet nadir of 6×10^3 platelets/L, the lowest in the study, but recovered rapidly (373×10^3 platelets/L by day 8) without platelet transfusion. No patient had cerebral malaria, although 3 had confusion and agitation, with 2 requiring sedation.

Predictors of Severe Malaria

Severe knowlesi malaria occurred in 27 of 57 (47%) patients aged ≥50 years compared with 11 of 73 (15%) aged <50 years ($P < .001$). However, using logistic regression, only parasite count and schizontemia >10% independently predicted severe malaria, after adjusting for age and excluding hyperparasitemia as a severity criterion (Table 6). The risk of severe knowlesi malaria increased 11-fold and 28-fold with parasitemias >20 000 parasites/μL and >100 000 parasites/μL, respectively (Table 7). The sensitivity and specificity for predicting severe disease using these thresholds were 75% and 79%, and 47% and 97%, respectively (Table 7), and the area under the ROC

Table 4. Details of All Patients With Severe Knowlesi Malaria

No.	Sex	Age (y)	Parasite Count (Parasites/μL)	Acute Kidney Injury[a]	Jaundice[b]	Hypotension[c]	Anemia[d]	Metabolic Acidosis[e]	Respiratory Distress[f]	Significant Abnormal Bleeding	Comments
1	F	62	55 769	Yes (Cr 282 μmol/L)	Yes (bilirubin 201 μmol/L)	Yes (BP 54 mm Hg)	No	No	No	No	ICU admission, on inotropes for 5 days. Severe ecchymoses. Cr 103 μmol/L on transfer (day 5).
2	M	61	284 559	No	Yes (bilirubin 57 μmol/L, Cr 201 μmol/L)	No	No	No	No	No	
3	M	20	3486	No	No	No	No	No	No	Yes (severe epistaxis)	Past history of ITP. Platelet nadir 6×10^3/μL, normalized by discharge.
4	M	32	151 160	No	Yes (bilirubin 45 μmol/L, Cr 145 μmol/L)	No	No	No	No	No	Complications occurred on day 1
5	M	42	61 778	No	Yes (bilirubin 67 μmol/L, Cr 215 μmol/L)	Yes (BP 70 mm Hg)	No	Yes ($HC0_3$ 12.4 mmol/L)	Yes (SaO_2 88%)	No	ICU admission, ARDS, on inotropes 2 days.
6	M	55	28 069	No	No	No	No	No	Yes (SaO_2 93%)	No	
7	M	38	153 734	No	Yes (bilirubin 82 μmol/L)	No	No	No	No	No	
8	M	55	304 454	No	Yes (bilirubin 52 μmol/L)	No	No	No	Yes (SaO_2 88%)	No	
9	M	33	25 874	No	Yes (bilirubin 48 μmol/L)	No	No	No	No	No	
10	M	55	327 062	No	Yes (bilirubin 279 μmol/L)	Yes (BP 70 mm Hg)	No	No	No	No	Confusion/agitation requiring sedation. ICU admission. Transfused day 2 for Hb 7.3 g/dL
11	F	60	3417	No	No	Yes (BP 80 mm Hg)	No	No	Yes (SaO_2 92%)	No	
12	M	22	48 833	No	No	No	No	No	Yes (SaO_2 92%)	No	
13	M	50	139 793	Yes (Cr 319 μmol/L)	Yes (bilirubin 160 μmol/L)	No	No	No	Yes (SaO_2 91%)	No	Cr 100 μmol/L on discharge (day 6).
14	F	61	160 328	No	No	No	No	No	No	No	
15	M	66	237 648	No	Yes (bilirubin 145 μmol/L, Cr 221 μmol/L)	Yes (BP 80 mm Hg)	No	No	No	No	Confusion/agitation requiring sedation, ICU admission, and intubation for airway protection. Transfused day 1 for Hb 8.2 g/dL. Inotropes given.

Table 4 continued.

No.	Sex	Age (y)	Parasite Count (Parasites/μL)	Acute Kidney Injury[a]	Jaundice[b]	Hypotension[c]	Anemia[d]	Metabolic Acidosis[e]	Respiratory Distress[f]	Significant Abnormal Bleeding	Comments
16	F	59	31 862	No	No	Yes (BP 66 mm Hg)	No	No	Yes (SaO_2 93%)	No	On inotropes 2 days
17	F	72	113 530	No	No	Yes (BP 76 mm Hg)	No	No	No	No	
18	M	62	16 632	No	No	Yes (BP 80 mm Hg)	No	No	Yes (SaO_2 91%)	No	Respiratory distress and hypotension developed day 1. Inotropes given day 2.
19	M	58	66 769	No	Yes (bilirubin 106 μmol/L, Cr 156 μmol/L)	No	No	No	No	No	
20	M	53	2523	No	Yes (bilirubin 43 μmol/L, Cr 252 μmol/L)	No	No	No	No	No	
21	M	51	26 368	Yes (Cr 267 μmol/L)	Yes (bilirubin 187 μmol/L)	Yes (BP 80 mm Hg)	No	No	Yes (SaO_2 93%)	No	Cr 103 μmol/L on discharge (day 5)
22	M	52	93 948	No	Yes (bilirubin 55 μmol/L)	No	No	No	No	No	
23	M	59	6669	No	No	Yes (BP 70 mm Hg)	No	No	No	No	
24	M	53	6450	Yes (Cr 268 μmol/L)	No	No	No	No	No	Yes (bleeding gastric ulcer)	Complications developed on day 3. Transfused day 2 for Hb 7.5 g/dL. Cr 97 μmol/L on discharge (day 7).
25	M	50	5522	No	No	Yes (BP 80 mm Hg)	No	No	No	No	Cr 189 μmol/L, bilirubin 39 μmol/L
26	F	69	168 279	No	No	No	No	No	Yes (SaO_2 91%)	No	Respiratory distress developed on day 1
27	M	22	108 041	No	Yes (bilirubin 58 μmol/L)	No	No	No	No	No	
28	M	67	32	Yes (Cr 617 μmol/L)	Yes (bilirubin 52 μmol/L)	No	No	Yes ($HC0_3$ 12.4 mmol/L)	Yes (SaO_2 93%)	No	Transfused day 2 for Hb 7.0 g/dL. Cr 132 μmol/L on discharge (day 6)
29	M	74	36 173	No	Yes (bilirubin 49 μmol/L, Cr 138 μmol/L)	Yes (BP 80 mm Hg)	No	No	No	No	
30	M	44	584 015	Yes (Cr 367 μmol/L)	No	No	No	No	No	No	Cr 176 μmol/L on admission, with peak occurring day 3. Cr 225 μmol/L and 117 μmol/L on days 27 and 66, respectively.
31	M	66	123 590	No	No	No	No	No	No	No	

Table 4 continued.

No.	Sex	Age (y)	Parasite Count (Parasites/μL)	Acute Kidney Injury[a]	Jaundice[b]	Hypotension[c]	Anemia[d]	Metabolic Acidosis[e]	Respiratory Distress[f]	Significant Abnormal Bleeding	Comments
32	M	68	118 064	No	No	No	No	No	No	No	Cr 173 μmol/L, confusion/ agitation.
33	M	49	249 994	No	Yes (bilirubin 182 μmol/L, Cr 170 μmol/L)	No	No	No	No	No	Transfused days 1 and 2 for Hb 7.8 g/dL. Hemoglobinuria day 1.
34	F	54	340 954	No	No	No	No	Yes ($HC0_3$ 12.8 mmol/L)	Yes (SaO_2 87%)	No	Cr 230 μmol/L. Transfused day 2 for Hb 7.2 g/dL.
35	M	47	34 779	Yes (Cr 850 μmol/L)	No	No	Yes (Hb 6.9 g/dL on day 8)	No	No	No	Hb nadir occurred day 11. Cr 590 μmol/L on day of discharge (day 11) and 144 μmol/L on day 30. Hemodialyzed days 1, 3, 5. ICU admission.
36	F	55	21 700	No	No	Yes (BP 70 mm Hg)	No	No	No	No	
37	M	62	506 218	Yes (Cr 580 μmol/L)	Yes (bilirubin 250 μmol/L)	No	Yes (Hb 6.5 g/dL on day 1)	Yes ($HC0_3$ 13.6 mmol/L)	Yes (SaO_2 68%)	No	Previous splenectomy. ARDS. Hemodialyzed until day 9. Cr 460 μmol/L on day of discharge (day 14); 121 μmol/L on day 29.
38	M	45	292 007	Yes (Cr 309 μmol/L)	Yes (bilirubin 49 μmol/L)	No	No	No	Yes (SaO_2 92%)	No	Cr 136 μmol/L on day of discharge (day 6)

No patient had hypoglycemia, coma, or multiple convulsions.

Abbreviations: ARDS, acute respiratory distress syndrome (partial pressure of arterial oxygen:fraction of inspired oxygen < 200 mm Hg); BP, systolic blood pressure; Cr, creatinine; Hb, hemoglobin; HCO_3, bicarbonate; ICU, intensive care unit; ITP, idiopathic thrombocytopenic purpura; SaO_2, oxygen saturation.

[a] Creatinine level >265 μmol/L.

[b] Bilirubin >43 μmol/L with creatinine level >132 μmol/L or density count >20 000 parasites/μL.

[c] Systolic blood pressure ≤80 mm Hg.

[d] Hemoglobin <7.0 g/dL.

[e] Bicarbonate <15 mmol/L.

[f] Respiratory rate >30 and oxygen saturation <94%.

Table 5. Details of All Patients With Severe Vivax Malaria

No.	Sex	Age	Parasite Count (Parasites/μL)	Jaundice[a]	Hypotension[b]	Respiratory Distress[c]	Significant Abnormal Bleeding	Multiple (>2) Convulsions	Comments
1	M	14	5488	No	Yes (BP 73 mm Hg)	No	No	Yes (3 convulsions)	
2	M	52	16 608	No	Yes (BP 70 mm Hg)	No	No	No	Blood cultures positive for *Streptococcus pneumoniae*
3	M	15	10 243	No	No	No	Yes (epistaxis & recurrent gum bleeding)	No	Platelet count 24 000/μL
4	M	54	84 403	Yes (bilirubin 118 μmol/L, Cr 166 μmol/L)	No	Yes (SaO_2 91%)	No	No	Treated initially with chloroquine. Respiratory distress developed day 1
5	M	43	460	No	Yes (BP 69 mm Hg)	No	No	No	
6	M	39	40 895	Yes (bilirubin 68 μmol/L)	Yes (BP 75 mm Hg)	No	No	No	
7	M	79	4387	No	Yes (BP 76 mm Hg)	No	No	No	GCS 12 on presentation

No patient had acute kidney injury, severe anemia, hypoglycemia, GCS score <11, or metabolic acidosis.

Antibiotics were given to patients 1, 2, 6, and 7. Preantibiotic blood cultures were negative in patients 6 and 7, but not done in patients 1, 3, 4, and 5.

Abbreviations: BP, systolic blood pressure; Cr, creatinine; GCS, Glasgow Coma Scale; SaO_2, oxygen saturation.

[a] Bilirubin >43 μmol/L with creatinine level >132 μmol/L or density count >20 000 parasites/μL.

[b] Systolic blood pressure ≤80 mm Hg.

[c] Respiratory rate >30 and oxygen saturation <94%.

curve was 0.8173 (Supplementary Figure). In vivax malaria, increasing age, but not parasitemia, was associated with severe disease, while in falciparum only parasitemia predicted severity. In multiple logistic regression controlling for referral (Table 6), *P. knowlesi* was associated with a 3-fold greater risk of severity than *P. falciparum* ($P = .020$).

P. knowlesi parasite count was associated with age ($\rho = 0.37$, $P < .001$), with median parasitemia higher in those ≥50 years (17 834 parasites/μL) versus <50 years (5430 parasites/μL, $P = .004$). This association was not seen with falciparum or vivax malaria. *Plasmodium knowlesi* parasitemia was higher among farmers and plantation workers compared with other occupations (median [interquartile range], 18 740 [4833–61 778] vs 4900 [1610–21 700] parasites/μL, $P = .001$), remaining significant after controlling for age ($P = .014$), and with no difference in fever duration.

Associations between parasitemia and laboratory variables, including platelet nadir, neutrophil count, bilirubin level, and lactate level, were stronger in *P. knowlesi* than in other species (Supplementary Table). Adjusting for age, knowlesi parasitemia was also associated with jaundice, respiratory distress (Table 8), and abdominal pain (odds ratio, 1.25 [log increase], $P = .026$). No association occurred between *P. knowlesi* parasitemia and fever duration. Controlling for age and parasitemia, patients with severe knowlesi malaria had higher neutrophil counts than those with nonsevere disease (Table 2); neutrophil count was associated with creatinine ($P = .012$) and development of respiratory distress ($P = .014$).

Response to Treatment

Nearly all (119/130, 92%) patients with knowlesi malaria were treated with oral ACT (artemether-lumefantrine = 109; artesunate-mefloquine = 10), with ≥1 dose of intravenous artesunate also given to 36 of 38 (95%) and 39 of 92 (42%) patients with severe and nonsevere knowlesi malaria, respectively. Fifteen (12%) received concurrent empirical antibiotics. Eleven (8%) patients with nonsevere knowlesi malaria received chloroquine with or without primaquine, including 7 misdiagnosed by microscopy as having *P. vivax*. All but 2 patients with falciparum malaria received oral ACT; all falciparum patients with severe malaria and 55 of 109 (50%) with nonsevere malaria received ≥1 dose of intravenous artesunate. Five patients with severe vivax malaria received intravenous artesunate. Median parasite clearance time (PCT) for all species was 2 days, with 55 (42%), 34 (28%), and 19 (44%) patients with knowlesi, falciparum, and vivax malaria, respectively, being smear-negative by

Table 6. Predictors of Severe Malaria[a]

	Univariate Analysis		Multivariate Analysis	
Variable	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Knowlesi malaria (n = 122)				
Age, years	1.04 (1.01–1.07)	.003	1.02 (.98–1.05)	.295
Male sex	1.27 (.51–3.16)	.604		
Parasite count (log)	2.18 (1.60–2.96)	<.0001	2.01 (1.44–2.79)	<.0001
Schizontemia >10%	8.07 (2.56–25.46)	<.0001	5.33 (1.44–19.80)	.012
Referred from district hospital	2.55 (1.01–6.44)	.047	1.21 (.38–3.81)	.750
Farmer or plantation worker	2.13 (.97–4.68)	.059		
Falciparum malaria (n = 130)				
Age	1.01 (.98–1.05)	.515		
Male sex	0.61 (.18–2.00)	.413		
Parasite count (log)	2.17 (1.37–3.43)	.001		
Referred from district hospital	2.87 (.88–9.38)	.081		
Farmer or plantation worker	1.74 (.35–5.55)	.351		
Vivax malaria (n = 43)				
Age	1.05 (1.00–1.11)	.041		
Male sex[b]	2.69 (.28–24.75)	.382		
Parasite count (log)	1.57 (.81–3.07)	.182		
Referred from district hospital	3.13 (.53–18.29)	.206		
Farmer or plantation worker	0.23 (.03–2.14)	.199		
Knowlesi and falciparum malaria (n = 252)				
Knowlesi malaria	3.09 (1.54–6.18)	.001	2.96 (1.19–7.38)	.020
Age	1.04 (1.02–1.06)	<.0001	1.02 (.99–1.04)	.169
Male sex	1.02 (.50–2.07)	.959		
Parasite count (log)	2.09 (1.63–2.68)	<.0001	2.06 (1.59–2.68)	<.0001
Referred from district hospital	3.31 (1.63–6.72)	.001	1.55 (.67–3.58)	.306

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Three patients with knowlesi malaria and hyperparasitemia as a sole severity criterion were considered nonsevere for this analysis.

[b] All 7 patients with severe vivax malaria were male; this analysis assumes 1 female has severe vivax malaria.

day 1 ($P = .032$). Two knowlesi patients had a PCT >3 days, both with severe malaria, including 1 splenectomized patient with a PCT of 9 days. Median fever clearance time was 1 day in knowlesi and vivax malaria and 2 days in falciparum malaria; median duration of hospitalization was 3 days for each species. No deaths occurred. Twenty-three (18%) knowlesi malaria patients were followed up during days 26–41, with no recurrences identified. One patient treated with artemether-lumefantrine was readmitted on day 42 with recurrent knowlesi malaria.

DISCUSSION

Plasmodium knowlesi was the commonest cause of severe malaria at QEH, and was associated with a 3-fold greater risk of severity than *P. falciparum*. Referral criteria for all species were identical, and the species difference in severity risk remained after adjusting for district referral. Parasite biomass was the major independent risk factor for severe knowlesi malaria, indicating the importance of early diagnosis and rapidly parasiticidal treatment given its 24-hour replication cycle. This study demonstrates excellent early efficacy of intravenous artesunate and ACT for severe and uncomplicated knowlesi malaria, respectively, with standardized early referral and standardized (including prereferral) use of intravenous artesunate and oral ACT likely contributing to the very low case-fatality rate.

The proportion of *P. knowlesi* cases with severe disease, both before (29%) and after (16%) exclusion of district hospital referrals, is similar to those previously reported (39% retrospectively at QEH [7] and 10% at a district hospital [3]). However, in contrast to previously reported case-fatality rates of 2% (district hospital) [3] and 11% (referral hospital) [7], no deaths occurred in our study. In previous reports, most knowlesi patients were treated with chloroquine for uncomplicated malaria and intravenous quinine for severe malaria [2, 3, 6, 7, 13].

Table 7. Risk of Severe Knowlesi Malaria, by Parasite Count

Parasite Count (Parasites/μL)	Total Patients, No.	Severe Malaria[a], No. (%, 95% CI)	OR (95% CI)	*P* Value	ROC Analysis (Sensitivity, Specificity)
<20 000	85	8 (9.4, 4.2–1.8)	Ref		
20 000–<35 000	12	5 (42, 19–68)	6.88 (1.35–31.98)	.002	
35 000–<50 000	6	2 (33, 4.3–78)	4.81 (.37–39.39)	.129	
50 000–<100 000	9	2 (22, 2.9–60)	2.75 (.24–18.06)	.244	
≥100 000	18	15 (83, 56–96)	48.1 (9.96–292.3)	<.0001	
<20 000	85	8 (9.4, 4.2–1.8)	Ref		
≥20 000	45	24 (53, 38–68)	11 (3.99–32.0)	<.0001	75.0%, 78.6%
<35 000	97	13 (13, 7.3–22)	Ref		
≥35 000	33	19 (58, 39–75)	8.77 (3.24–23.9)	<.0001	59.4%, 85.7%
<50 000	103	15 (15, 8.4–23)	Ref		
≥50 000	27	17 (63, 42–81)	9.97 (3.47–29.0)	<.0001	53.1%, 89.8%
<100 000	112	17 (15, 9.1–23)	Ref		
≥100 000	18	15 (83, 56–96)	27.9 (6.65–160)	<.0001	46.9%, 96.9%

[a] Three patients with hyperparasitemia as a sole severity criterion, and 3 patients with bilirubin >43 μmol/L plus parasite count >20 000/μL as a sole severity criterion, were considered nonsevere for this analysis.

Abbreviations: CI, confidence interval; OR, odds ratio; ROC, receiver operating characteristic.

While chloroquine is efficacious for uncomplicated knowlesi malaria [14], in a retrospective study parasite clearance was faster with artemether-lumefantrine and artesunate compared with chloroquine and quinine, respectively, with fewer deaths with artesunate than quinine [7].

The low case-fatality rate in this study is not due to more liberal severe malaria criteria. We used stricter criteria for severe knowlesi malaria than those previously used [3], based on modified WHO 2010 criteria for severe falciparum malaria [9, 10]. Furthermore, deaths from severe knowlesi malaria continued to occur at hospitals in Sabah during 2010–2011 where intravenous artesunate was delayed or not given [8].

In this study >80% of patients with *P. knowlesi* parasitemia >100 000 parasites/μL met other severity criteria, supporting the use of this cutoff as a *P. knowlesi* severity criterion for research purposes and mandating intravenous artesunate. The choice of parasitemia threshold to guide a clinical recommendation for the use of intravenous artesunate below this cutoff is less clear; however, in settings where other (including laboratory) severity criteria cannot be reliably assessed, a conservative cutoff of >20 000 parasites/μL may be appropriate.

Consistent with previous reports [1, 3], knowlesi patients were older than those with falciparum or vivax malaria. Although older patients with knowlesi malaria were at greater risk of severe disease, this association was explained by the strong correlation between age and parasite count. A greater risk of hyperparasitemia among older patients has also been reported with falciparum malaria [15].

The lower median parasitemia in nonsevere knowlesi malaria relative to nonsevere *P. falciparum* indicates a lower fever threshold with *P. knowlesi* than with *P. falciparum*, suggesting a greater inflammatory response per parasitized red

Table 8. Association Between Parasite Count and Complications

	Plasmodium knowlesi		*Plasmodium falciparum*	
Complication	OR (95% CI), Log Increase in Parasite Count	*P* Value	OR (95% CI), Log Increase in Parasite Count	*P* Value
Acute kidney injury	1.53 (1.04–2.25)	.032	1.78 (.87–3.68)	.117
Jaundice[a]	2.26 (1.56–3.28)	<.0001[b]	4.07 (1.87–8.86)	<.0001[b]
Respiratory distress	1.65 (1.18–2.30)	.004[b]	1.54 (.82–2.90)	.175
Hypotension	1.49 (1.07–2.06)	.017	1.36 (.82–2.27)	.237
Metabolic acidosis	1.31 (.78–2.22)	.308	1.71 (.90–3.24)	.102

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] With parasite count >20 000 parasites/μL (*P. knowlesi* and *P. vivax*) or >100 000 parasites/μL (*P. falciparum*), and/or creatinine level >132 μmol/L.

[b] Remains significant ($P < .05$) after adjusting for age.

cell, as seen with *P. vivax* [16, 17]. In keeping with this, neutrophil count correlated with *P. knowlesi* parasitemia, and was greater in severe disease.

In total, 84 cases of severe knowlesi malaria have been reported: 38 in this series and 46 in 6 previous reports [2, 3, 6–8, 13]. There has been no case of knowlesi-associated coma. Coma has previously been reported in up to half of Asian adults with severe falciparum malaria [18, 19], involving microvascular sequestration of parasitized erythrocytes [20, 21], ICAM-1–mediated cytoadherence [21] to brain endothelial cells, and reduced red cell deformability. Although *P. knowlesi*–infected erythrocytes have been shown to bind to ICAM-1 in vitro [22], the single *P. knowlesi* autopsy report did not detect ICAM-1 on brain endothelium, despite demonstrating cerebral parasite accumulation [6]. Red cell agglutination and sludging underlie fatal knowlesi malaria in monkey models [23, 24]. Different microvascular pathogenic mechanisms between *P. knowlesi* and *P. falciparum* seem likely.

Although ARDS occurred in 10 of 22 (45%) patients with severe knowlesi malaria in the retrospective QEH series [7], it was rare in this prospective study, occurring in only 2 patients with neither requiring mechanical ventilation. This may reflect earlier use of artesunate and faster parasite clearance. Milder respiratory distress (hypoxemia without ARDS) was more common, occurring in approximately 30% of patients with severe malaria; the association with parasitemia and neutrophilia suggests parasite-induced inflammatory lung injury.

Thrombocytopenia is near-universal in knowlesi malaria [1, 3, 7]; however, the mechanism is unknown. The absence of thrombocytopenia in 2 asplenic knowlesi malaria patients contrasts with reports of thrombocytopenia in splenectomized patients with falciparum malaria [25–28], suggesting differences in splenic pathophysiology between species.

As previously reported [3, 7, 13], AKI was common in severe knowlesi malaria. The mechanisms are unknown. Creatinine was associated with parasitemia and neutrophil count. In the single autopsy report of fatal knowlesi malaria, acute tubular necrosis was seen, as well as numerous parasitized erythrocytes within glomerular capillaries. As in falciparum AKI [29], both parasite biomass and inflammation are likely important.

Plasmodium vivax was also associated with severe malaria in a significant proportion of patients (16%), supporting the now large body of evidence documenting severe complications from this species [30–36]. Complications of *P. vivax* included hypotension, jaundice, respiratory distress, and multiple convulsions, with risk of severity associated with increasing age.

Our study had several limitations. A greater proportion of patients with *P. knowlesi* than other species were referred from district hospitals. Although introducing a potential bias, this was controlled for in analysis. The high proportion of referred patients with knowlesi malaria limited our ability to quantitate the true incidence of complications. We were also unable to assess all patients prior to commencement of treatment.

We confirm the public health importance of *P. knowlesi* in Sabah, with this species now the commonest cause of severe malaria at the state's largest hospital, and with a greater risk of severity than *P. falciparum*. With the marked reductions in falciparum and vivax malaria incidence in Sabah over the last 20 years [37], loss of cross-species immunity may contribute to rising incidence and severity of disease from *P. knowlesi* [5]. The low case-fatality rate from severe knowlesi malaria in this series contrasts with the high case-fatality rate previously reported with the use of nonartemisinin therapies [7]. Early referral protocols and standardized (including prereferral) use of intravenous artesunate and oral ACT likely contributed to the low case-fatality rate from *P. knowlesi* and indeed all *Plasmodium* species in this series, and warrant wider implementation.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank all the patients and clinical staff involved in their care; Rita Wong, Beatrice Wong, and Ann Wee for assistance with clinical and laboratory study procedures; Nadine Kurz for assistance with performing the polymerase chain reaction assays; Ferryanto Chalfein for performing microscopy; and Kim Piera, Melissa Gallop, and Ella Curry for laboratory and logistical support. We also thank the Director General of Health (Malaysia) for permission to publish this study.

Financial support. This work was supported by the Australian National Health and Medical Research Council (program grant number 496600, fellowships to N. M. A. and T. W. Y., and scholarship to B. E. B.).

Potential conflicts of interest. All authors: No reported conflicts. 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.

References

1. Barber BE, William T, Jikal M, et al. *Plasmodium knowlesi* malaria in children. Emerg Infect Dis **2011**; 17:814–20.
2. Cox-Singh J, Davis TM, Lee KS, et al. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis **2008**; 46:165–71.
3. Daneshvar C, Davis TM, Cox-Singh J, et al. Clinical and laboratory features of human *Plasmodium knowlesi* infection. Clin Infect Dis **2009**; 49:852–60.
4. Joveen-Neoh WF, Chong KL, Wong CM, Lau TY. Incidence of malaria in the Interior Division of Sabah, Malaysian Borneo, based on nested PCR. J Parasitol Res **2011** [Epub ahead of print] .
5. Cox-Singh J. Zoonotic malaria: *Plasmodium knowlesi*, an emerging pathogen. Curr Opin Infect Dis **2012**; 25:530–6.

6. Cox-Singh J, Hiu J, Lucas SB, et al. Severe malaria—a case of fatal *Plasmodium knowlesi* infection with post-mortem findings. Malar J **2010**; 9:10.
7. William T, Menon J, Rajahram G, et al. Severe *Plasmodium knowlesi* malaria in a tertiary hospital, Sabah, Malaysia. Emerg Infect Dis **2011**; 17:1248–55.
8. Rajahram G, Barber BE, William T, Menon J, Anstey NM, Yeo TW. Deaths due to *Plasmodium knowlesi* malaria in Sabah, Malaysia: association with reporting as *P. malariae* and delayed parenteral artesunate. Malar J **2012**; 11:284.
9. World Health Organization. Guidelines for the treatment of malaria. 2nd ed. Geneva, Switzerland: WHO; **2010**.
10. Hien TT, Day NPJ, Phu NH, et al. A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. N Engl J Med **1996**; 335:76–83.
11. Imwong M, Tanomsing N, Pukrittayakamee S, Day NPJ, White NJ, Snounou G. Spurious amplification of a *Plasmodium vivax* small-subunit RNA gene by use of primers currently used to detect *P. knowlesi*. J Clin Microbiol **2009**; 47:4173.
12. Padley D, Moody A, Chiodini P, Saldanha J. Use of a rapid, single-round, multiplex PCR to detect malarial parasites and identify the species present. Ann Trop Med Parasitol **2003**; 97:131–7.
13. Lee CE, Adeeba K, Freigang G. Human *Plasmodium knowlesi* infections in Klang Valley, peninsular Malaysia: a case series. Med J Malaysia **2010**; 65:63–5.
14. Daneshvar C, Davis TM, Cox-Singh J, et al. Clinical and parasitological response to oral chloroquine and primaquine in uncomplicated human *Plasmodium knowlesi* infections. Malar J **2010**; 9:238.
15. Dondorp AM, Lee SJ, Faiz M, et al. The relationship between age and the manifestations of and mortality associated with severe malaria. Clin Infect Dis **2008**; 47:151.
16. Yeo TW, Lampah DA, Tjitra E, et al. Greater endothelial activation, Weibel-Palade body release and host inflammatory response to *Plasmodium vivax*, compared with *Plasmodium falciparum*: a prospective study in Papua, Indonesia. J Infect Dis **2010**; 202:109–12.
17. Ross R, Thomson D. Some enumerative studies on malarial fever. Proc R Soc Lond B Biol Sci **1910**; 83:159–73.
18. Dondorp A, Pongponratn E, White N. Reduced microcirculatory flow in severe falciparum malaria: pathophysiology and electron-microscopic pathology. Acta Trop **2004**; 89:309–17.
19. Yeo TW, Lampah DA, Gitawati R, et al. Impaired nitric oxide bioavailability and L-arginine–reversible endothelial dysfunction in adults with falciparum malaria. J Exp Med **2007**; 204:2693–704.
20. Pongponratn E, Turner GDH, Day NPJ, et al. An ultrastructural study of the brain in fatal *Plasmodium falciparum* malaria. Am J Trop Med Hyg **2003**; 69:345–59.
21. Turner GDH, Morrison H, Jones M, et al. An immunohistochemical study of the pathology of fatal malaria: evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. Am J Pathol **1994**; 145:1057.
22. Fatih FA, Siner A, Ahmed A, et al. Cytoadherence and virulence—the case of *Plasmodium knowlesi* malaria. Malar J **2012**; 11:33.
23. Knisely MH, Stratman-Thomas WK. Knowlesi malaria in monkeys; microscopic pathological circulatory physiology of rhesus monkeys during acute *Plasmodium knowlesi* malaria. J Natl Mal Soc **1945**; 4:285.
24. Knisely MH, Stratman-Thomas WK. Microscopic observations of intravascular agglutination of red cells and consequent sludging of blood in rhesus monkeys infected with knowlesi malaria. Anat Rec **1948**; 101:701.
25. Demar M, Legrand E, Hommel D, Esterre P, Carme B. *Plasmodium falciparum* malaria in splenectomized patients: two case reports in French Guiana and a literature review. Am J Trop Med Hyg **2004**; 71:290–3.
26. Pauzner R, Goldschmied-Reouven A, Hay I, et al. Delayed parasite clearance in a splenectomized patient with falciparum malaria who was treated with artemisinin derivatives. Clin Infect Dis 1997:923–5.
27. Looareesuwan S, Suntharasamai P, Webster HK, Ho M. Malaria in splenectomized patients: report of four cases and review. Clin Infect Dis **1993**; 16:361.
28. Bachmann A, Esser C, Petter M, et al. Absence of erythrocyte sequestration and lack of multicopy gene family expression in *Plasmodium falciparum* from a splenectomized malaria patient. PLoS One **2009**; 4:e7459.
29. Nguansangiam S, Day NPJ, Hien TT, et al. A quantitative ultrastructural study of renal pathology in fatal *Plasmodium falciparum* malaria. Trop Med Int Health **2007**; 12:1037–50.
30. Anstey NM, Douglas NM, Poespoprodjo JR, Price R. *Plasmodium vivax*: clinical spectrum, risk factors and pathogenesis. Adv Parasitol **2012**. In press.
31. Price RN, Douglas NM, Anstey NM. New developments in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance. Curr Opin Infect Dis **2009**; 22:430.
32. Tjitra E, Anstey NM, Sugiarto P, et al. Multidrug-resistant *Plasmodium vivax* associated with severe and fatal malaria: a prospective study in Papua, Indonesia. PLoS Med **2008**; 5:e128.
33. Genton B, D'Acremont V, Rare L, et al. *Plasmodium vivax* and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. PLoS Med **2008**; 5:e127.
34. Kochar DK, Das A, Kochar SK, et al. Severe *Plasmodium vivax* malaria: a report on serial cases from Bikaner in northwestern India. Am J Trop Med Hyg **2009**; 80:194–8.
35. Barcus MJ, Basri H, Picarima H, et al. Demographic risk factors for severe and fatal vivax and falciparum malaria among hospital admissions in northeastern Indonesian Papua. Am J Trop Med Hyg **2007**; 77:984–91.
36. Lacerda MVG, Fragoso SCP, Alecrim MGC, et al. Postmortem characterization of patients with clinical diagnosis of *Plasmodium vivax* malaria: to what extent does this parasite kill? Clin Infect Dis **2012**.
37. World Health Organization. World Malaria Report 2011. Geneva, Switzerland: WHO; **2011**. Available at: http://www.who.int/malaria/world_malaria_report_2011/9789241564403_eng.pdf. Accessed 10 July 2012.