The Treatment of Plasmodium knowlesi Malaria

Article in Trends in Parasitology · October 2016

CITATIONS
4

READS
141

5 authors, including:

Bridget Barber
Menzies School of Health Research
38 PUBLICATIONS  575 CITATIONS

Matthew J Grigg
Menzies School of Health Research and Charl...
39 PUBLICATIONS  505 CITATIONS

Timothy William
Queen Elizabeth Hospital, Kota Kinabalu, Mal...
68 PUBLICATIONS  828 CITATIONS
Plasmodium knowlesi occurs across Southeast Asia and is the most common cause of malaria in Malaysia. High parasitaemias can develop rapidly, and the risk of severe disease in adults is at least as high as in falciparum malaria. Prompt initiation of effective treatment is therefore essential. Intravenous artesunate is highly effective in severe knowlesi malaria and in those with moderately high parasitaemia but otherwise uncomplicated disease. Both chloroquine and artemisinin-combination therapy (ACT) are highly effective for uncomplicated knowlesi malaria, with faster parasite clearance times and lower anaemia rates with ACT. Given the difficulties with microscope diagnosis of P. knowlesi, a unified treatment strategy of ACT for all Plasmodium species is recommended in coendemic regions.

Emergence of P. knowlesi Malaria

The simian parasite P. knowlesi was first identified in 1932 [1]; however, it was not recognized as an important cause of zoonotic malaria until 2004, when Singh et al. reported a large focus of knowlesi malaria in Sarawak, Malaysia, in patients previously misdiagnosed with the microscopically near-identical Plasmodium malariae [2]. Notifications of human knowlesi malaria in Malaysia have increased substantially since this time, with P. knowlesi now the most common cause of malaria in this country [3,4] and threatening the otherwise successful progress towards malaria elimination [5]. The simian reservoir and mosquito vectors for P. knowlesi are found in Southeast Asia, with over 500 million people theoretically at risk of infection [6]. Human infections have been reported in all countries in Southeast Asia except Timor Leste and Laos, and in travellers returning from this region [6]. P. knowlesi infection can rapidly progress to high parasitaemias, and in adults the risk of severe disease is at least as high as that of falciparum malaria [7]. Thus, prompt diagnosis and initiation of effective treatment is essential for preventing morbidity and mortality.

In this review we discuss the treatment of uncomplicated and severe knowlesi malaria, highlighting the importance of a unified treatment strategy of artemisinin-based treatment for all Plasmodium species in knowlesi-endemic areas.

Diagnosis of P. knowlesi Malaria

While microscopy is sensitive for the diagnosis of P. knowlesi in clinical disease, the mature trophozoites and schizonts of P. knowlesi resemble those of P. malariae, and the two species cannot be reliably differentiated [8]. Laboratory reporting of P. knowlesi as the more benign P. malariae has been associated with failure to recognize severe malaria and consequent delayed initiation of parenteral therapy, with fatal outcomes [9]. In areas of significant endemicity for P. knowlesi, parasites with the microscopic appearance of P. malariae should be reported as P. knowlesi and treated as such. In Sabah, Malaysia, it is likely that the inclusion of P. knowlesi in laboratory microscopy reporting has contributed to increased recognition and more timely treatment.

© 2016 Elsevier Ltd. All rights reserved.
administration of intravenous artesunate for severe knowlesi malaria, and to the recent reduction in *P. knowlesi* case-fatality rates [3].

By microscopy, *P. knowlesi* may also resemble *P. falciparum* due to similarity between the ring forms of the two species [8], and misdiagnosis as *Plasmodium vivax*, and vice versa, is also common [10]. In Sabah, where *P. malariae/P. knowlesi* accounted for 76% of all microscopy-based malaria notifications in 2014, PCR-confirmed *P. knowlesi* accounted for 21% and 38% of blood films diagnosed as *P. falciparum* and *P. vivax*, respectively [11], highlighting the limitations of microscopy for species differentiation in regions where *P. falciparum*, *P. vivax*, and *P. knowlesi* all commonly occur.

Rapid diagnostic tests (RDTs) are insensitive for *P. knowlesi* malaria, particularly at low parasite densities, and in general are unable to distinguish *P. knowlesi* from *P. vivax* [12,13]. Thus, while microscopy remains the standard diagnostic method for clinical purposes, treatment decisions, guidelines, and surveillance must take into account the potential inaccuracies of microscopy, and molecular methods such as PCR are required to confirm the diagnosis of knowlesi malaria. Confirmation of species by PCR will also allow administration of the antihypnozoite drug primaquine to patients with *P. vivax* (or *P. ovale*) malaria, who may have been misdiagnosed by microscopy as *P. knowlesi* [10].

**In vitro Sensitivity of *P. knowlesi* to Antimalarials**

In the only study to date evaluating ex vivo drug susceptibility of *P. knowlesi*, clinical isolates were obtained from Sarawak, Malaysia. Growth inhibition by artemisinin, artemether, artesunate, dihydroartemisinin, chloroquine, and mefloquine was assessed by using a modified schizont maturation assay [14]. However, this study included only six clinical isolates, and the stage specificity of the drugs tested, an important confounder of susceptibility of nonfalciparum *Plasmodium*, was not assessed [15,16]. This may explain, in part, why the synchronised laboratory *P. knowlesi* H strain appeared more sensitive to chloroquine (median 50% inhibitory concentrations [IC₅₀] 3.2 nM, range 2.2–4.7 nM) than the nonsynchronised clinical isolates (median IC₅₀ 23 nM, range 11–38 nM), although with the latter IC₅₀S still well below the 100 nM threshold used to define *P. falciparum* chloroquine resistance [17]. Both the clinical isolates and the H strain of *P. knowlesi* were reported to be highly sensitive to artesinin and its derivatives [14], with IC₅₀S <2.2 nM, lower than reported IC₅₀S of artemisinins against *P. falciparum* [18–20]. *P. knowlesi* isolates were reported to be less sensitive to mefloquine than to chloroquine or artesinin [14].

**Drug Resistance Mutations**

If, as is likely, *P. knowlesi* malaria occurs primarily as a zoonosis [21,22], drug-selected mutations would not be expected to occur. In the six Malaysian clinical isolates above, Fatih et al. analysed the *P. knowlesi* orthologues of the *P. falciparum* chloroquine-resistance transporter (CRT) and multidrug resistance protein 1 (MDR1) genes, associated with susceptibility of *P. falciparum* to chloroquine and mefloquine, respectively, and did not detect mutations associated with drug resistance [14]. In keeping with this, Tyagi et al. evaluated the CRT and dihydrofolate reductase (DHFR) sequences of 53 *P. knowlesi* clinical isolates from the Andaman and Nicobar islands, and found all to be wild type, with the latter suggesting lack of drug pressure from pyrimethamine [23]. These findings have been extended by Grigg et al., who analysed the DHFR sequences of over 400 *P. knowlesi* isolates from Sabah, Malaysia, and demonstrated multiple polymorphisms but no evidence of drug selection [24]. Similarly, Assefa et al. analysed 48 *P. knowlesi* isolates from Sarawak, Malaysia, and found no evidence of drug-selected mutations in the CRT, MDR1, DHFR, dihydropteroate synthase (DHPS), and kelch K13 genes [25], with the latter two associated with *P. falciparum* resistance to sulfadoxine and artesinin derivatives, respectively. Although Pinheiro et al. reported dimorphism and polymorphism among *P. knowlesi* isolates in
the multidrug-resistance-associated protein MRP1 and the multidrug-resistance protein MDR2 genes, both members of the ATP-binding cassette transporter family of genes associated with mefloquine resistance in *P. falciparum* [26], the functional implications in *P. knowlesi* are not clear.

**Antimalarial Agents for the Treatment of Uncomplicated *P. knowlesi* Malaria**

**Chloroquine**

Until recently, *P. knowlesi* was widely misdiagnosed as *P. malariae* due to microscopic near-identity [2, 8]. As such, *P. knowlesi* in Malaysia was almost uniformly treated with chloroquine, which was until recently the recommended treatment of *P. malariae* in Malaysia. Initial studies demonstrated chloroquine to be highly effective. In the first report of a large focus of human infections with *knowlesi* malaria, Singh et al. reported 92 patients with *knowlesi* malaria treated with chloroquine (25 mg/kg over 3 days) at a district hospital in Sarawak, with primaquine given at 24 and 48 h [2]. Ten patients also received a single dose of sulfadoxine/pyrimethamine. Severity status was not reported, although one patient had a parasite count >100 000 parasites/μL. Median parasite clearance time was 2.4 days (range 1–5 days) and no deaths were reported.

The efficacy of chloroquine for the treatment of uncomplicated *knowlesi* malaria was further demonstrated in a prospective observational study involving adults at the same district hospital [27]. Chloroquine was given for 3 days with primaquine at 24 and 48 h. Patient characteristics and parasite clearance kinetics in this (and other studies reported from *knowlesi*-endemic areas) are shown in Table 1. Chloroquine plus primaquine was found to be highly efficacious. Mean time to 50% (PCT50) and 90% (PCT90) reduction of parasitaemia at presentation was 3.1 h and 10.3 h, respectively [27]. All patients had cleared their parasites by day 3, and all were negative by PCR on days 7, 14, 21, and 28. Similar efficacy of chloroquine was demonstrated by Grigg et al. in a randomized clinical trial involving children and adults with uncomplicated malaria at three district hospitals in Northeastern Sabah, Malaysia [28] (Table 1). Parasite clearance times with chloroquine in this study were not confounded by concurrent primaquine treatment, and were slower (PCT50 and PCT90 6.3 h and 14.8 h, respectively); nevertheless all patients had cleared their parasites by day 3, and there were no treatment failures during the 42 days of follow up. In both studies chloroquine appeared less effective against ring and early trophozoite stages compared to late trophozoites, with an increasing proportion of ring-stage parasites post-treatment despite reduction in overall parasitaemia, as assessed by microscopy. The initial parasite reduction ratio was lower with chloroquine than with artesunate-mefloquine, with the difference reflecting the higher frequency of a transient increase in parasitaemia with chloroquine [28]. The safety of chloroquine in patients with uncomplicated malaria and parasite counts >20 000 has not been adequately established [7].

**Artemisinin-Combination Treatment (ACT)**

A variety of oral artemisinin-combination therapies have been successfully used for the treatment of uncomplicated *knowlesi* malaria, including artesunate-mefloquine [28], artemether-lumefantrine [7, 29], and dihydroartemisinin-piperaquine [30].

The best data exist for artesunate-mefloquine, which was compared to chloroquine in the only randomized clinical trial in *knowlesi* malaria reported to date [28]. In this study, artesunate-mefloquine was highly efficacious, with faster parasite clearance than with chloroquine (Table 1). PCT50 and PCT90 for artesunate-mefloquine were 3.4 and 8.9 h respectively, with 84% of patients aperasitemic by 24 h compared to 55% of patients treated with chloroquine (Table 1). The difference in parasite clearance was particularly marked for ring-stage parasites (PCT50 8.6 h and 13.8 h for artesunate-mefloquine and chloroquine, respectively, P < 0.01; and PCT90 11.7 hours and 18.9 hours, respectively, P < 0.0001). As with chloroquine, there were no early or late treatment failures. These results are consistent with an earlier nonrandomized study
Table 1. Series of Antimalarial Chemotherapy Used in the Treatment of *Plasmodium knowlesi* Malaria in Adults and Children in Endemic Areas

<table>
<thead>
<tr>
<th>Study design</th>
<th>Number of patients</th>
<th>Per cent male</th>
<th>Median (range)</th>
<th>Median (range) parasites/μL</th>
<th>PCT50 (h), median</th>
<th>PCT90 (h), median</th>
<th>Parasite reduction ratio at 24 h (% 95%CI)</th>
<th>% negative at 24 h</th>
<th>Time to parasite clearance (&lt;5/μL), median (range)</th>
<th>Died</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uncomplicated malaria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroquine and primaquine Prospective observational</td>
<td>33</td>
<td>58</td>
<td>46</td>
<td>3.724 (1845–7480)</td>
<td>3.1 (range 2.8–3.4)</td>
<td>10.3 (range 9.4–11.4)</td>
<td>99.4 (97.0–99.9)</td>
<td>33</td>
<td>35.8 (30.8–51.2) h</td>
<td>0</td>
<td>[27]</td>
</tr>
<tr>
<td>Chloroquine Randomised controlled trial</td>
<td>125</td>
<td>75</td>
<td>32 (7–85)</td>
<td>1329 (33–35 873)</td>
<td>6.3 (95%CI 5.1–7.5)</td>
<td>14.8 (95%CI 13.4–16.1)</td>
<td>97.5 (96.6–98.4)</td>
<td>55</td>
<td>24 (6–60) h</td>
<td>0</td>
<td>[28]</td>
</tr>
<tr>
<td>Chloroquine Retrospective</td>
<td>16</td>
<td>50</td>
<td>9 (4–14)</td>
<td>2240 (200–14 400)</td>
<td>NR</td>
<td>NR</td>
<td>6+</td>
<td>2 days (range 1–6)</td>
<td>2 days (range 1–6)</td>
<td>0</td>
<td>[64]</td>
</tr>
<tr>
<td>Artesunate/ mefloquine Randomised controlled trial</td>
<td>115</td>
<td>81</td>
<td>33 (3–82)</td>
<td>1457 (36–35 008)</td>
<td>3.4 (95%CI 2.9–4.0)</td>
<td>8.9 (95%CI 8.2–9.6)</td>
<td>99.8 (99.7–100)</td>
<td>84</td>
<td>18 (6–48) h</td>
<td>0</td>
<td>[28]</td>
</tr>
<tr>
<td>Artesunate-lumefantrine Prospective observational</td>
<td>27</td>
<td>70</td>
<td>31 (17–70)</td>
<td>4061 (252–60 840)</td>
<td>NR</td>
<td>NR</td>
<td>33+</td>
<td>2 days (range 1–3)</td>
<td>1 day (range 0–3)</td>
<td>0</td>
<td>[7]</td>
</tr>
<tr>
<td>Artesunate-lumefantrine Retrospective</td>
<td>6</td>
<td>100</td>
<td>30</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>66+</td>
<td>6+ (range 0–3)</td>
<td>1 day (range 0–3)</td>
<td>0</td>
<td>[29]</td>
</tr>
<tr>
<td>Oral quinine Retrospective</td>
<td>11</td>
<td>83</td>
<td>45</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>12+</td>
<td>2.5 days (range 1–3)</td>
<td>0</td>
<td>[29]</td>
<td></td>
</tr>
<tr>
<td><strong>Severe malaria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous artesunate Prospective observational</td>
<td>36</td>
<td>75</td>
<td>55 (20–74)</td>
<td>100 995 (32–584 015)</td>
<td>NR</td>
<td>NR</td>
<td>33+</td>
<td>NR</td>
<td>0 days (range 1–3)</td>
<td>0</td>
<td>[7]</td>
</tr>
<tr>
<td>Intravenous artesunate Retrospective</td>
<td>6</td>
<td>63</td>
<td>56</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>50%</td>
<td>2 days (range 1–3)</td>
<td>1 (17%)</td>
<td>[29]</td>
<td></td>
</tr>
<tr>
<td>Intravenous quinine Prospective observational</td>
<td>10</td>
<td>30</td>
<td>64 (36–73)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>2 (20%)</td>
<td>[27]</td>
<td></td>
</tr>
<tr>
<td>Intravenous quinine Retrospective</td>
<td>16</td>
<td>69</td>
<td>55</td>
<td>4+</td>
<td>NR</td>
<td>NR</td>
<td>30%</td>
<td>4 days (range 2–7)</td>
<td>5 (27%)</td>
<td>[29]</td>
<td></td>
</tr>
</tbody>
</table>

*Based on daily routine hospital microscopy.

*Supplementary data.

NR, not recorded.
demonstrating efficacy of artesunate-mefloquine for uncomplicated malaria at a tertiary referral hospital in Sabah [7].

Mefloquine is recommended by the WHO only as an artemisinin partner drug, with use of mefloquine monotherapy discouraged [31]. Successful treatment of uncomplicated knowlesi malaria with mefloquine monotherapy has been reported [32,33]; however, treatment failures have been reported in rhesus macaques [34,35], and in a single human case of knowlesi malaria with high parasitaemia [36]. However, as with other agents [3,9,37], the treatment failure in this latter case may relate to the administration of oral therapy for a high parasitaemia infection, rather than the recommended intravenous therapy (see below).

One case of mefloquine-related psychosis and suicidal ideation was reported in the clinical trial of artesunate-mefloquine vs. chloroquine [28]. Mefloquine has been used extensively for the treatment of uncomplicated falciparum and vivax malaria and its neuropsychiatric effects have been well documented, with serious events occurring in 1 in 1217 Asian adults [38]. While artesunate-mefloquine is approved by the Malaysian Ministry of Health as an alternative ACT for uncomplicated knowlesi malaria in Sabah, ACTs with less risk of serious adverse events are available.

Although no randomised controlled trials have been reported to date, artemether-lumefantrine is listed by the Malaysian Ministry of Health as the preferred ACT for the treatment of uncomplicated knowlesi malaria and is widely used. In a prospective nonrandomised observational study at a tertiary referral hospital in Sabah, 27 patients with uncomplicated knowlesi malaria were safely and effectively treated with artemether-lumefantrine; while parasite clearance kinetics were not fully reported, all patients had cleared their parasites by day 3 (Table 1) [7]. In a previous retrospective study at the same hospital, patients with uncomplicated knowlesi malaria treated with artemether-lumefantrine had faster parasite clearance times than those treated with chloroquine or oral quinine [29].

Use of dihydroartemisinin-piperaquine has been reported in a single case involving a patient with uncomplicated knowlesi malaria in Kalimantan, Indonesia [30]. Parasitaemia fell from 1.25% to <0.01% within 24 h, and was undetectable by day 3.

Other Agents
Atovaquone-proguanil, although not widely available in knowlesi-endemic areas, has been used for the treatment of uncomplicated knowlesi malaria in returned travellers (Table 2), with rapid recovery reported in all cases [39–42].

Rationale for Unified Treatment Strategy of ACT for All Uncomplicated Malaria
While chloroquine remains extremely efficacious for the treatment of uncomplicated knowlesi malaria, artesunate-mefloquine is associated with faster fever-clearance time, faster parasite-clearance time, reduced risk of post-treatment anaemia, and, in Malaysia, reduced bed-occupancy due to the Ministry of Health policy of hospitalising patients until they have negative blood films on two consecutive days [28]. For these reasons, malaria treatment guidelines in Malaysia have recently been updated to recommend artemether-lumefantrine as first-line asexual-stage treatment for uncomplicated malaria due to *P. knowlesi* and all other *Plasmodium* species.

The major rationale behind a unified ACT regimen for all species in coendemic regions is the superior efficacy of ACT over chloroquine against multidrug-resistant *P. falciparum* and *P. vivax* in this region [43]. Use of chloroquine for the treatment of microscopy-diagnosed knowlesi malaria is associated with a risk of administering chloroquine to misdiagnosed *P. falciparum* or
<table>
<thead>
<tr>
<th>Country of residence</th>
<th>Region where infection acquired</th>
<th>Age/sex</th>
<th>Parasitaemia</th>
<th>Severity status</th>
<th>Treatment</th>
<th>Outcome/comments</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>Ko Payam, West Thailand</td>
<td>45 M</td>
<td>0.8%</td>
<td>Uncomplicated</td>
<td>Chloroquine</td>
<td>Recovered</td>
<td>[68]</td>
</tr>
<tr>
<td>Sweden</td>
<td>Bario Highlands, Sarawak, Malaysia</td>
<td>35 M</td>
<td>0.1%</td>
<td>Uncomplicated</td>
<td>Mefloquine</td>
<td>Recovered. Discharged afebrile after 2 days</td>
<td>[32]</td>
</tr>
<tr>
<td>United States</td>
<td>Palawan, Phillipines</td>
<td>50 F</td>
<td>2.9%</td>
<td>Not stated</td>
<td>Atovaquone-proguanil and primaquine</td>
<td>Recovered</td>
<td>[69]</td>
</tr>
<tr>
<td>Singapore</td>
<td>Perak, West Malaysia</td>
<td>11 M</td>
<td>Not stated</td>
<td>Uncomplicated</td>
<td>Oral quinine, changed to chloroquine</td>
<td>Recovered. Discharged home after 5 days</td>
<td>[70]</td>
</tr>
<tr>
<td>Australia</td>
<td>Kalmantan, Indonesia</td>
<td>39 M</td>
<td>185 parasites/µL</td>
<td>Uncomplicated</td>
<td>Atovaquone-proguanil</td>
<td>Recovered</td>
<td>[40]</td>
</tr>
<tr>
<td>New Zealand</td>
<td>Bintulu, Sarawak, Malaysia</td>
<td>40 M</td>
<td>Not stated</td>
<td>Uncomplicated</td>
<td>Atovaquone-proguanil, changed to artemether-lumefantrine</td>
<td>Recovered</td>
<td>[41]</td>
</tr>
<tr>
<td>Finland</td>
<td>West Malaysia</td>
<td>53 M</td>
<td>&lt;1%</td>
<td>Uncomplicated</td>
<td>Intravenous quinine, followed by oral quinine and doxycycline</td>
<td>Recovered. Treatment complicated by hypoglycaemia, and an episode of mild visual and hearing loss</td>
<td>[71]</td>
</tr>
<tr>
<td>Taiwan</td>
<td>Palawan, Phillipines</td>
<td>60 M</td>
<td>Not stated</td>
<td>Uncomplicated</td>
<td>Chloroquine</td>
<td>Recovered</td>
<td>[72]</td>
</tr>
<tr>
<td>Spain</td>
<td>Southeast Asia (likely West Malaysia)</td>
<td>39 M</td>
<td>250 parasites/µL</td>
<td>Uncomplicated</td>
<td>None</td>
<td>Recovered</td>
<td>[73]</td>
</tr>
<tr>
<td>Japan</td>
<td>West Malaysia</td>
<td>35 M</td>
<td>10 120 parasites/µL</td>
<td>Uncomplicated</td>
<td>Mefloquine</td>
<td>Recovered. PCT 40 h, FCT 28 h. Discharged on day 7; no relapses during 5 months follow-up</td>
<td>[33]</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Kapit, Sarawak, Malaysia</td>
<td>38 M</td>
<td>84 000 parasites/µL</td>
<td>Severe (bilirubin 99 µmol/L with parasite count &gt;20 000/µL)</td>
<td>Chloroquine</td>
<td>Recovered. PCT 40 h</td>
<td>[46]</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Borneo, Malaysia</td>
<td>32 F</td>
<td>0.0005%</td>
<td>Uncomplicated</td>
<td>Atovaquone-proguanil</td>
<td>Recovered. PCT 3 days</td>
<td>[74]</td>
</tr>
<tr>
<td>Germany</td>
<td>Ranong Province, Thailand</td>
<td>42 M</td>
<td>920 parasites/µL</td>
<td>Uncomplicated</td>
<td>Atovaquone-proguanil</td>
<td>Recovered. Discharged on day 4</td>
<td>[42]</td>
</tr>
<tr>
<td>Germany</td>
<td>Ranong Province, Thailand</td>
<td>52 F</td>
<td>0.01%</td>
<td>Uncomplicated</td>
<td>Artemether-lumefantrine</td>
<td>Recovered. Discharged on day 3</td>
<td>[75]</td>
</tr>
<tr>
<td>Germany</td>
<td>Myanmar or Thailand</td>
<td>73 M</td>
<td>3%</td>
<td>Severe (ARDS, shock, acidosis, renal failure, jaundice)</td>
<td>Intravenous quinine and oral doxycycline</td>
<td>PCT 48 h. Admission further complicated by ventilator-associated pneumonia. Required haemodialysis for 5 weeks. Discharged after 5 weeks</td>
<td>[49]</td>
</tr>
<tr>
<td>Germany</td>
<td>Phuket, Thailand</td>
<td>54 M</td>
<td>473 parasites/µL</td>
<td>Uncomplicated</td>
<td>Atovaquone-proguanil</td>
<td>Recovered. Parasitaemia 11 parasites/µL at 48 h. Patient also newly diagnosed with HIV</td>
<td>[39]</td>
</tr>
<tr>
<td>Germany</td>
<td>Southern Thailand</td>
<td>55 F</td>
<td>0.2%</td>
<td>Severe (acute kidney injury developed following admission)</td>
<td>Intravenous artesunate then artemether-lumefantrine</td>
<td>Recovered. PCT 2 days, creatinine normalised by day 4</td>
<td>[50]</td>
</tr>
</tbody>
</table>

*Abbreviations: ARDS, acute respiratory distress syndrome; F, female; FCT, fever clearance time; M, male; PCT, parasite clearance time.
*Based on clinical and laboratory data provided in the case reports.
*Patient had taken prophylaxis with mefloquine and atovaquone-proguanil. Chloroquine given after PCR results revealed P. knowlesi, 2 months after discharge from hospital.
**P. vivax.** Misdiagnosis of knowlesi malaria by microscopy is common, with *P. knowlesi* frequently misdiagnosed as *P. vivax* or *P. falciparum*, and vice versa [10]. Chloroquine is ineffective for the treatment of falciparum malaria throughout Southeast Asia, and chloroquine-resistant *P. vivax* is also highly prevalent in this region, particularly in Indonesia and Malaysia [44,45]. In a recent clinical trial involving 103 Malaysian adults and children with vivax malaria, the risk of recurrent parasitaemia by day 28 was 61% in patients treated with chloroquine [44]. In contrast, artesunate-mefloquine was highly effective, with rapid *P. vivax* clearance and no treatment failures. This led to ACT being recommended over chloroquine as first-line treatment of uncomplicated malaria from all *Plasmodium* species in Malaysia. A unified treatment strategy of ACT for all uncomplicated malaria would be similarly advantageous in other regions coendemic for *P. knowlesi*, *P. falciparum*, and *P. vivax*, with the addition of primaquine radical cure for *P. vivax* (and *P. ovale*) malaria.

An additional advantage of a unified ACT treatment strategy is that it reduces the risk of inadvertent chloroquine administration to patients with unrecognized severe knowlesi malaria. Although there are isolated reports of successful use of chloroquine for the treatment of severe knowlesi malaria [2,46], fatal outcomes have been reported with its use [3,37]. Although use of oral ACT has been associated with a fatal outcome in a patient with a very high (>200 000/μL) parasitaemia [3] and patients with severe malaria should receive intravenous artesunate (see below), it is possible that the broader stage specificity and more rapid parasite clearance of oral ACT may be associated with improved outcomes if used inadvertently in those with recognised high parasitaemia.

**Primaquine**

*P. knowlesi* does not form hypnozoites and hence antirelapse therapy is not required. Sexual stages are cleared rapidly with drugs used to treat asexual-stage infection, without use of primaquine [28]. *P. knowlesi* gametocytes are cleared with nonprimaquine antimalarial agents. The proportion of patients with gametocytemia, as evaluated by qPCR, is reduced from 85% to 6% and 4% 7 days after treatment with chloroquine and artesunate-mefloquine respectively, with transmissibility of these residual gametocytes likely to be limited [28]. With *P. knowlesi* existing primarily as a zoonosis without evidence for substantial human–human transmission [21,22], administration of primaquine as a transmission-reducing agent in endemic regions is not considered necessary, particularly in areas where testing for glucose-6-phosphate dehydrogenase (G6PD) deficiency is not available. Primaquine is indicated in cases where confirmatory PCR identifies a true *P. vivax* infection misdiagnosed as *P. knowlesi*.

**Treatment of Severe *P. knowlesi* Malaria**

High parasitaemias can develop rapidly following *P. knowlesi* infection and lead to the complications of severe malaria. Thus, prompt recognition of severe malaria and initiation of appropriate treatment is essential for preventing morbidity and mortality. Severe knowlesi malaria has been defined according to modified criteria for severe falciparum malaria (Box 1), with a lower parasite count of 100 000 parasites/μL used as the cut-off to define hyperparasitaemia [7,27]. In adults, the most common manifestations of severe knowlesi malaria include acute kidney injury, hyperparasitaemia, jaundice, shock, and respiratory distress [6]. Metabolic acidosis occurs less commonly but has been reported in most fatal cases [3,9,37]. In contrast to falciparum malaria, coma does not appear to be a feature of severe knowlesi malaria, with no case of coma, with exclusion of other causes, yet reported in PCR-confirmed knowlesi malaria. Also in contrast to falciparum malaria, severe disease from *P. knowlesi* has not been reported in children, and no paediatric deaths have been reported [3]. Nearly all cases of severe knowlesi malaria have been reported from Malaysia; however, severe knowlesi malaria has also been reported in Thailand [47] and Brunei [48], and in travellers returning from endemic regions [46,49,50] (Table 2).
The WHO recommends treatment with intravenous artesunate for severe malaria of any species [31], based on large randomized controlled trials in Southeast Asian adults and children [51] and African children [52] demonstrating reduced mortality with intravenous artesunate compared to intravenous quinine for the treatment of severe falciparum malaria. Intravenous artesunate has also been shown to be highly effective for the treatment of severe knowlesi malaria. In a prospective study at a tertiary referral hospital in Sabah, intravenous artesunate was administered to 38 patients with severe knowlesi malaria, with zero mortality [7]. In an earlier retrospective study conducted at the same hospital, before and after the introduction of intravenous artesunate in 2010 [29], 5 of 16 (31%) patients with severe knowlesi malaria treated with intravenous quinine died, compared to 1 of 6 (17%) patients treated with intravenous artesunate. In another prospective observational study in Sarawak, 2 of 10 (20%) patients with severe knowlesi malaria and treated with intravenous quinine died [27]. This difference in case-fatality rates is reflected in a recent state-wide study in Sabah, demonstrating that the fatality rate from knowlesi malaria fell from 9.2/1000 case notifications in 2010 to 1.6/1000 case notifications in 2014 [3]. It is likely that this reduction in mortality is due at least in part to the increasing use of intravenous artesunate for severe malaria.

While intravenous artesunate should clearly be administered to patients with clinical or laboratory evidence of severe knowlesi malaria, and to those not tolerating oral medications (Box 1), the optimal treatment for patients with moderately high parasite counts in the absence of other evidence of severe disease is less certain. In a recent prospective study, 53% of patients with a parasite count of >20 000 parasites/μL had severe disease, compared to only 9% of patients with a parasite count of <20 000 [7]. The risk of severe knowlesi malaria increased 11-fold and 28-fold with parasitaemias >20 000 parasites/μL and >100 000 parasites/μL, respectively [7]. In another study, there was a 64% chance of severe disease in patients with a parasite count above 35 000 parasites/μL [53]. In the randomized controlled study by Grigg et al., a small number of patients with parasite counts of between 20 000/μL and 35 000 parasites/μL were safely treated with both chloroquine and mefloquine-artesunate [28]. However, this small number of patients did not allow confirmation of the safety of oral therapy at this level of parasitaemia. Thus, the requirement for intravenous therapy for patients with parasite counts of 20 000–100 000/μL in the absence of other clinical or laboratory evidence of severe disease remains uncertain.

### Box 1. Indications for Parenteral Therapy with Artesunate in *Plasmodium knowlesi* Malaria

- Inability to tolerate oral intake
- Parasite count >20 000/μL and unable to assess other clinical and/or laboratory criteria for severe malaria
- Severe malaria (Table I)

### Table I. Criteria for Severe *Plasmodium knowlesi* Malaria

<table>
<thead>
<tr>
<th>Condition</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrovable coma</td>
<td>Glasgow Coma Scale &lt;11</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td>Oxygen saturation &lt;92% with respiratory rate &gt;30 breaths/min</td>
</tr>
<tr>
<td>Shock</td>
<td>Systolic blood pressure &lt;80 mmHg with cool peripheries or impaired capillary refill</td>
</tr>
<tr>
<td>Jaundice</td>
<td>Bilirubin &gt;50 μmol/L, with parasitaemia &gt;20 000/μL and/or creatinine &gt;132 μmol/L</td>
</tr>
<tr>
<td>Severe anaemia</td>
<td>Haemoglobin &lt;7.0 g/dL (adults)</td>
</tr>
<tr>
<td></td>
<td>Haemoglobin &lt;5.0 g/dL (children)*</td>
</tr>
<tr>
<td>Significant abnormal bleeding</td>
<td>Blood glucose &lt;2.2 mmol/L</td>
</tr>
<tr>
<td>Hypoglycaemia</td>
<td>Blood glucose &lt;2.2 mmol/L</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td>Bicarbonate &lt;15 mmol/L or lactate &gt;5 mmol/L</td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>Creatinine &gt;265 μmol/L</td>
</tr>
<tr>
<td>Hyperparasitaemia</td>
<td>Parasite count &gt;100 000/μL (or &gt;2% or infected red blood cells)</td>
</tr>
</tbody>
</table>

*Not reported to date in knowlesi malaria.*
recognition of this uncertainty, the WHO recommends that patients with knowlesi malaria and parasitaemia >20,000/μL receive intravenous artesunate if clinical or laboratory criteria for severe disease cannot be rapidly or adequately assessed [54].

For patients fulfilling criteria for severe knowlesi malaria, intravenous artesunate should generally be given for a minimum of 3 doses (2.4 mg/kg), 12 h apart [54]. This should be followed by a complete course (3 days) of oral ACT such as artemether-lumefantrine once oral intake is tolerated, according to local guidelines and availability. For patients commenced on intravenous artesunate in the absence of criteria for severe malaria, oral ACT should be substituted as soon as oral intake is tolerated.

In severe falciparum malaria intravenous artesunate has in some cases been associated with post-treatment haemolysis, presenting as severe anaemia 7 to 14 days after treatment [55,56]. While early haemolysis with haemoglobinuria has been reported following artesunate use in severe knowlesi malaria [57], post-artesunate delayed haemolysis has not yet been reported in P. knowlesi infection. WHO recommends that all patients who receive intravenous artesunate be monitored for delayed haemolytic anaemia [31].

**Adjunctive and Supportive Therapy**

There have been no clinical trials of adjunctive therapy in severe knowlesi malaria. However, no adjunctive therapies have proven effective in severe falciparum malaria, and none are recommended for use in severe malaria from either species [54]. Severe knowlesi malaria is frequently associated with multiorgan failure requiring intensive supportive management. Supportive treatment for severe knowlesi malaria should be according to management guidelines for severe falciparum malaria, including inotropic and ventilatory support [29], haemodialysis for acute kidney injury [7,29], and blood transfusions as required [31,54]. Thrombocytopenia is universal in severe knowlesi malaria and significant abnormal bleeding, although uncommon, has been reported [7,37,48,58,59]. Platelet counts recover rapidly after commencement of antimalarial treatment [7]. While platelet transfusions have been given for bleeding associated with severe thrombocytopenia, the clinical benefits are uncertain. There have been no clinical trials to guide intravenous fluid management in knowlesi malaria. In severe falciparum malaria liberal administration of intravenous fluids has been shown to be deleterious [60,61] and conservative fluid regimens have been shown to be safe [62]. Because the risk of acute respiratory distress syndrome and noncardiogenic pulmonary oedema is at least as high in severe knowlesi malaria as in severe falciparum malaria [29,54], careful use of conservative intravenous fluid regimens would appear prudent [60].

There are no clinical trials to guide the use of empirical antibiotics in severe knowlesi malaria. Concurrent bacteremia, predominantly Gram negative, occurs in up to 13% of adults with severe falciparum malaria [62] and Enterobacter bacteremia has been reported in two series of severe knowlesi malaria [7,29]. Empirical intravenous broad-spectrum antibiotics are commonly administered in severe knowlesi malaria with multiorgan failure until blood cultures are negative [7].

**Treatment of P. knowlesi Malaria in Children**

Although Plasmodium knowlesi is the most common cause of childhood malaria in Malaysia [3], prospective studies reporting the clinical features of knowlesi malaria in children are yet to be published. However, while mild to moderate anaemia and thrombocytopenia are common, the multiorgan failure that characterises severe disease in adults does not appear to occur in children, nor the severe anaemia commonly seen with falciparum and vivax malaria in this age-group [63]. Indeed, there have been no cases of severe paediatric PCR-confirmed knowlesi malaria reported to date [6,64]. The treatment recommendations for knowlesi malaria in children are the same as for adults. In the randomized controlled trial by Grigg et al., 12 and 8 children
(defined as <12 years old) were treated with artesunate-mefloquine and chloroquine, respectively [28]. Median (range) parasite counts in these children were 377 (36–9998) parasites/μL and 2024 (459–20 750) respectively, and all were aparasitaemic by 48 h [28]. The use of chloroquine in an additional 16 children with uncomplicated knowlesi malaria was reported in a retrospective study; median parasite clearance time was 2 days, although a parasite clearance time of 5 days was reported in an 8-year-old boy with an admission parasitaemia of 14 400 parasites/μL [64]. Successful use of oral and intravenous quinine for knowlesi malaria in children was also reported [64]. In the absence of reported severe knowlesi malaria in children, the use of intravenous artesunate for this indication has not been described, but would be recommended based on its known efficacy in children with severe falciparum malaria [51,52], and in adults with severe knowlesi malaria [7].

Treatment of *P. knowlesi* Malaria in Pregnancy

In contrast to *P. falciparum* and *P. vivax*, *P. knowlesi* infection during pregnancy appears to be relatively rare, with only five cases reported to date [29,65]. However, despite its rarity, adverse maternal and infant outcomes have been reported, including severe maternal malaria, foetal loss and low birth weight [29,65], and effective treatment is essential. Any woman with severe malaria in pregnancy should be treated with immediate intravenous artesunate, regardless of species and including those in the first trimester [31]. Women with uncomplicated knowlesi malaria in the second or third trimester of pregnancy may be treated with oral ACT, as per the guidelines for treatment of uncomplicated falciparum malaria in pregnancy [31]. In the first trimester of pregnancy uncomplicated knowlesi malaria may be treated with chloroquine. If there is any doubt as to the microscopic diagnosis, alternative treatments include quinine and clindamycin, as per guidelines for falciparum and vivax malaria [31]. In falciparum malaria large studies have found no increased risk of stillbirths, miscarriage, or major congenital malformations with artemisinin regimens compared to nonartemisinin regimens [66,67]. Accordingly the WHO Malaria Policy Advisory Committee has recommended that WHO update their 2015 Guidelines to consider the timely inclusion of ACT as a first-line therapeutic option for falciparum malaria in the first trimester of pregnancy, and artemether-lumefantrine may also therefore be an alternative therapy for microscopy-diagnosed first trimester knowlesi malaria where there is doubt about the diagnosis.

Concluding Remarks

*P. knowlesi* malaria occurs primarily as a zoonosis, and appears therefore free from drug-resistance mutations developing as a result of human antimalarial use. Thus, while prompt administration of intravenous artesunate is mandatory for severe disease, a range of antimalarials is available for uncomplicated knowlesi malaria. Chloroquine remains highly effective for uncomplicated disease. However, artemisinin-based treatments are associated with a superior early therapeutic response and less anaemia. Moreover, treatment of uncomplicated knowlesi malaria must take into account the possibility of misdiagnosed chloroquine-resistant *P. falciparum* or *P. vivax*, or unrecognized high parasitaemia, all of which would not be adequately treated with chloroquine. Thus, ACT should be considered the treatment of choice for uncomplicated knowlesi malaria, and in areas coendemic for chloroquine-resistant *P. vivax* and *P. falciparum*, there is a strong rationale for universal ACT for all species. The comparative efficacy of the various ACTs has not been evaluated, and warrants prospective clinical efficacy studies. In addition, given the strong correlation between parasite density and development of complications in knowlesi malaria, further studies are required to determine the safety of oral ACT in patients with moderately high parasitaemia (20 000–100 000/μL) but otherwise uncomplicated disease (see Outstanding Questions).

Acknowledgments

National Health and Medical Research Council of Australia (Fellowships to BEB, TWY, NMA; Scholarship to MJG).
Trends in Parasitology, Month Year, Vol. xx, No. yy

References

4. Yusof, R. et al. (2014) High proportion of kivostis malaria in recent malaria cases in Malaysia. Malaya J. 13, 168
33. Tanizaki, R. et al. (2013) First case of Plasmodium knowlesi infection in a Japanese traveller returning from Malaysia. Malaya J. 12, 1-4
34. Tripathi, R. et al. (2005) Mefloquine resistance reversal action of ketoconazole-a cytochrome P 450 inhibitor, against mefloquine-resistant malaria. Parasitolology 130, 475-479
42. Mackroth, M.S. et al. (2016) Rapid-antigen test negative malaria in a traveller returning from Thailand; molecularly diagnosed as Plasmodium knowlesi. Open Forum Infect. Dis. 3, doi:009

49. Sellmaier, M. et al. (2014) Severe Plasmodium knowlesi infection with multi-organ failure imported to Germany from Thailand/Myan-
mar. Malānā J 13, 422


51. Donkor, A. et al. (2009) South East Asian Quinine Artesunate Malana Trial (SEAQUAMAT) group, Artesunate versus quinine for

52. Donkor, A.M. et al. (2010) Artesunate versus quinine in the
treatment of severe falciparum malaria in African children (AQUA-
MAT): an open-label, randomised trial. Lancet 376, 1647–1657

53. Willmann, M. et al. (2012) Laboratory markers of disease severity in
Plasmodium knowlesi infection: a case control study. Malānā J. 11, 363


Blood 124, 167–175

56. Gómez-Juryent, J. et al. (2015) Delayed haemolysis after artesu-
nate therapy in a cohort of patients with severe imported malaria
http://dx.doi.org/10.1016/j. eimc.2015.11.003

57. Barber, B.E. et al. (2016) Intravascular haemolysis with haemo-
globinuria in a splenectomized patient with severe Plasmodium
knowlesi malaria. Malānā J. 15, 462


59. Lee, C.E. et al. (2010) Human Plasmodium knowlesi infections in

60. Hanson, J. et al. (2014) The fluid management of adults with
severe malaria. Crit. Care 18, 1–9

61. Hanson, J.P. et al. (2013) Fluid resuscitation of adults with severe falciparum malaria: effects on acid-base status, renal function, and
extravascular lung water. Crit. Care Med. 41, 972–981

ment strategy in adults hospitalised with malaria. PLoS ONE 10, e0143062

63. Douglas, N.M. et al. (2013) Major burden of severe anemia from
non-falciparum malaria species in Southern Papua: a hospital-
based surveillance study. PLoS Med. 10, e1001575

Emerg. Infect. Dis. 17, 814–820

65. Barber, B.E. et al. (2014) Plasmodium knowlesi malaria in preg-
nancy. J. Infect. Dis. 211, 1104–1110

exposure to artemisinin derivatives in the first trimester of preg-

67. Moore, K.A. et al. (2016) Safety of artemisinins in first trimester of
prospectively followed pregnancies: an observational study. Lan-
cet Infect. Dis. 16, 576–583


70. Fan, L. et al. (2013) Plasmodium knowlesi infection: a diagnostic
http://
dx.doi.org/10.1136/bcr-2013-009558


72. Kuo, M-C. et al. (2009) A case report of simian malaria, Plasmo-
dium knowlesi, in a Taiwanese traveler from Palawan Island, the
Philippines. TAIWAN EPIDEMOL. Bull. 25, 179–191

73. Tang, T. et al. (2010) First case of detection of Plasmodium
knowlesi in Spain by Real Time PCR in a traveler from Southeast
Asia. Malānā J. 9, 219

74. Link, L. et al. (2010) Molecular detection of Plasmodium knowlesi
in a Dutch traveler by real-time PCR. J. Clin. Microbiol. 50, 2523–
2524

infection) after traveling to Thailand.Dt. med. Wschr. 140, 815–817