*P. knowlesi* and other *Plasmodium* species causing malaria in Sabah, Malaysia: clinical spectrum and antimalarial efficacy

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**P. knowlesi** geographical distribution

- **Pk** - all countries in SEA
- Range of macaque hosts and *An. leucophyurus* group vector overlaps
- Microscopy Pk looks like Pm (and Pf or Pv) – only PCR valid
- Spreading Pf artemisinin resistance, widespread Pv CQ resistance
- Malaysia Pf / Pv malaria elimination goal for 2020

Cooper et al. unpublished 2018
Sabah district hospital prospective malaria study

- Kudat, Kota Marudu and Pitas district hospitals
- Positive microscopy for malaria
- PCR final species confirmation
- All ages
- Modified WHO severe malaria criteria
- 6 hourly blood slides
- 3 day hospital admission
- Follow-up at day 7, 14, 28, 42
Sabah district malaria study – clinical spectrum

<table>
<thead>
<tr>
<th></th>
<th>P. knowlesi</th>
<th>P. vivax</th>
<th>P. falciparum</th>
<th>P. malariae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>481</td>
<td>172</td>
<td>96</td>
<td>31</td>
</tr>
<tr>
<td>Children (%)</td>
<td>44 (9)</td>
<td>71 (41)</td>
<td>31 (32)</td>
<td>11 (36)</td>
</tr>
</tbody>
</table>

- *P. knowlesi* malaria patients;
  - Older
  - Lower parasitaemia
  - Lower risk of mild-moderate anaemia
  - Higher risk of thrombocytopenia

- Morbidity in **children**: anaemia (82%), AKI (26%), no severe disease

Grigg et al. CID 2018
**Sabah district malaria study – severe disease**

- **Severe** knowlesi malaria in 5.8%
- Severe knowlesi complications
  - AKI (10%)
  - Hyperparasitaemia (8%)
  - Anaemia (8%)
  - Jaundice (8%)
- No coma / cerebral malaria
- **Best predictor of severe** disease
  = parasite count >15,000/μL (aOR 16.1; NPV 98.5%; AUC 0.80; p<0.001)
- Adult case fatality rate of 0.5%
**Treatment of *P. knowlesi* malaria – ASMQ vs. CQ**

October 2012 to December 2015
229 enrolled (20 children)
115 treated with ASMQ
114 treated with Chloroquine

**Results:**

- AS-MQ has superior early response
  - Faster parasite clearance
    PCT = 18 vs. 24 h
    Neg 24 h = 84% vs. 55%
  - Faster fever clearance
    11 vs. 14 h
  - Earlier hospital discharge
    IRR 0.86
- No difference in Day 28 or 42 treatment outcomes
- Anaemia risk during follow-up lower with AS-MQ: 62% vs. 75%
  \( p=0.035 \)
- One neuropsychiatric SAE with ASMQ

Grigg et al. Lancet ID. 2016
Treatment of *P. knowlesi* malaria – ACT vs. CQ

**Figure 2.** Meta-analysis of ACT vs CQ for treatment of uncomplicated knowlesi malaria: difference in proportional parasite clearance at 24 h

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Treatment</th>
<th>RD (95% CI)</th>
<th>ACT</th>
<th>CQ</th>
<th>Weight %</th>
<th>P–value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT KNOW</td>
<td>2016</td>
<td>ASMQ vs CQ</td>
<td>0.29 (0.18, 0.41)</td>
<td>97/115</td>
<td>61/111</td>
<td>65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAN KNOW</td>
<td>2017</td>
<td>AL vs CQ</td>
<td>0.16 (0.00, 0.32)</td>
<td>44/58</td>
<td>39/65</td>
<td>35</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Overall (I–squared = 44.3%, p = 0.180)

Grigg et al. CID. 2018
Treatment of *P. vivax* malaria – ASMQ vs. CQ

October 2012 to December 2015
103 patients enrolled (38 children)
54 treated with ASMQ
49 treated with Chloroquine

**Primary endpoint** = risk of treatment failure by day 28 (modified ITT)

CQ arm = 61.8% (CI95 46.8-75.6)
ASMQ arm = 0%  \(p<0.001\)

**Early treatment failure**
CQ = 4/49 (8.2%; CI95 2.5-9.6)

**Late treatment failure**
CQ = 22/42 (52.4%; CI95 36.4-68.0)

*No additional treatment failures after day 28 post PQ*

Grigg et al. CID. 2016
Treatment of *P. malariae* malaria – ASMQ vs. CQ

Oct 2012 to Dec 2015

- 6 treated with ASMQ
- 4 treated with CQ
- 20 treated with ACT (ASMQ or AL)
- 11 treated with CQ

**Results:**

PCT: 24 h vs. 96 h

Neg at 48 h:
- 6/6 (100%) vs. 0/4 (0%)
- 19/19 (100%) vs. 5/9 (56%)

Neg at 72 h:
- 6/6 (100%) vs. 1/4 (25%)
- 19/19 (100%) vs. 7/9 (78%)

- No difference in anaemia at day 28: 1/9 (11%) vs. 0/6 (0%)

Unpublished data
**P. falciparum** artemisinin resistance surveillance study

**Oct 2012 to Dec 2015:**
- Therapeutic efficacy monitoring: n = 49
- Molecular drug resistance marker evaluation: n = 278

**Results:**

*No clinical evidence of delayed parasite clearance*

PCT: median 26 h (IQR 19-42)

$PCT_{50}$: **4.3 h** (IQR 1.9-8.4)

Neg at 72 h: 49 (100%)

Molecular markers: 20 SNPs in K13 propeller domain all wild-type

Manuscript in preparation
Policy changes from Sabah malaria studies

KKM (national)
• ACT for uncomplicated *P. knowlesi* malaria
• ACT for uncomplicated *P. vivax* malaria
• Barber et al. CID 2013
  • severe knowlesi malaria definition
  • referral guidelines
  • early artesunate treatment

WHO (international)
• Artesunate for all malaria species if severe including *P. knowlesi*
• RDTs not effective for *P. knowlesi*
• To be potentially included:
  • ACT first-line for uncomplicated knowlesi malaria
  • *Parasite count >15,000/ul = give initial dose artesunate*
HOT NORTH supported ongoing malaria studies

Effect of paracetamol on acute kidney injury in knowlesi malaria – PACKNOW

*HOT NORTH project grant 2017
• Dan Cooper – PhD
• Enrolments completed – 378 knowlesi malaria patients
• Laboratory assays to be finalised, initial data analyses commenced

Sabah state-wide drug resistance monitoring
• All *P. falciparum* patients from Sabah with PCR confirmation
• SpotMalaria Sanger SEA collaboration
• Updated K13 markers, geographic mapping of genotypes
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Noraziela Mohd binti Yassin
Jusia Deyeo
Kelly Nestor
Joseph Benedict
Mohd Rizan Osman

Sabah study participants

Study funders

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National Health and Medical Research Council, Australia

Monkeybar Project: UK Medical Research Council (ESEI grant)
Treatment of *P. knowlesi* malaria – anaemia

Anaemia risk during follow-up lower in AS-MQ arm

- 62% vs. 75%  \( p=0.035 \)

Anaemia developing during follow-up

- 7/27 (26%) in CQ arm, 0/26 (0%) in ASMQ (\( p=0.005 \))

Correlated with parasite clearance rate (\( k \))

Hb nadir at day 2 both arms

No patients with Hb <7 g/dL

No evidence PADH
Treatment of *P. knowlesi* malaria – gametocytes

- Pks25 RNA assay:
  - 85% (85/100) patients positive for gametocytes at day 0
  - 5 patients positive at day 7 post treatment – CQ arm (2/48), ASMQ arm (3/49)
  - CT values lower at day 7

- Microscopic gametocyte clearance times
  - AS-MQ and CQ arms (mean hours 7.2 vs. 7.9; \( p=0.715 \)).

- No patients positive for gametocytes at follow-up by microscopy
Treatment of *P. knowlesi* malaria – AL vs. CQ

Nov 2014 to Jan 2016
123 patients enrolled (18 children)
115 treated with AL
114 treated with Chloroquine

Grigg et al. CID. 2018

**Results:**

- PCT: 18 vs. 24 h; *p*=0.021
- Neg at 24 h: 76% vs. 60%; *p*=0.061
- Neg at 24 h (if pc>1000/uL): 63% vs. 40%; *p*=0.047
- Median mg/kg:
  - Artem = 8.7; Lum = 52.0
  - CQ = 24.8
- Anaemia = 67% vs. 81%
- Hospital discharge = 2414 vs 2800 days/1000 patients; IRR 0.86; *p*<0.001
**Early therapeutic response (ASMQ vs. CQ)**

Parasite clearance time
- 19 vs. 48 hours \( (p<0.001) \)

Slope of clearance curve
- 0.31 vs. 0.13 \( (p<0.001) \)

Negative at 72 hours
- 100% vs. 83.7% \( (p<0.002) \)

Bed-occupancy
- 4,037 versus 6,510 days/1,000 patients
  (incidence rate ratio 0.62; \( p<0.001 \)).

**Gametocyte prevalence at day 28**
- 0% vs. 23.8% \( (p<0.001) \)

**Risk of anaemia at day 28**
- 24.4% vs. 54.8% \( (P=0.004) \)
- AOR = 6.8
District hospital malaria studies - results

Morbidity in children

- 11-fold higher risk of anaemia at presentation to adults; 82% vs. 36%
- Similar risk of mild-moderate AKI to adults; 26% vs. 19%
- No severe disease

Grigg et al. CID 2018
Risk factors for people getting *P. knowlesi* malaria

October 2012 to Jan 2015

229 people with *P. knowlesi* malaria

683 healthy controls

**Increased risk**

- Activities near forest edge
- Male farmers
- Plantation workers
- History of clearing forest
- Travel to work/school in late afternoon
- Villages where monkeys are seen more often
- Sleeping outside when travelling (and not using bednet)

**Decreased risk**

- No thick forest around house
- Indoor residual spraying
- G6PD deficiency