Malaria in pregnancy and the neonate
Patophysiology, diagnosis, prevention and management

Eskild Petersen, MD
Adjungated Professor of Tropical Medicine
Institute for Clinical Medicine
Aarhus University

and
Senior Consultant Infectious Diseases
The Royal Hospital
Muscat, Oman

ProMED Moderator Parasitic Diseases
Editor
Human malaria

*Plasmodium falciparum*
- High mortality, drug resistance
- Persistent liverforms

*P. vivax*
- Persistent liverforms

*P. ovale*
- Classic and variant P.o

*P. malariae*
- Low pathogen, latent many years
- 24 hours cycle, confused with *P. malariae* in Southeast Asia

*P. knowlesi*
The Malaria Atlas project

Figure 2: Global distribution of infection with Plasmodium falciparum malaria
Reproduced from Hay and Snow, and licensed to the Malaria Atlas Project (http://www.map.ox.ac.uk). PfAPI=P. falciparum annual parasite incidence per 1000 people per year. PfPR<10<sub>P</sub>falciparum parasite rate age-standardised to 2-10 years.
Life-cycle
Exit from the liver

Release of merozoites to the bloodstream
Pathophysiology
Malaria disease

Day 0
Infective bite

Dag 5-7
The liver merozoites invade the blood

Day 10 – 14
Start of symptoms

Parasites per μl
- 500,000 (10%)
- 50,000 (1:100)
- 5000 (1:1000)
- 500 (1:10,000)
- 50 (1:100,000)
- 5 (1:1 mil.)

P.falciparum fever curve
P.vivax fever curve
P.falciparum parasitemia

Diagnostics difficult
7.1.1 | SEVERE FALCIPARUM MALARIA

For epidemiological purposes, severe falciparum malaria is defined as one or more of the following, occurring in the absence of an identified alternative cause and in the presence of P. falciparum asexual parasitaemia.

- **Impaired consciousness:** A Glasgow coma score < 11 in adults or Blantyre coma score < 3 in children
- **Prostration:** Generalized weakness so that the person is unable to sit, stand or walk without assistance
- **Multiple convulsions:** More than two episodes within 24 h
- **Acidosis:** A base deficit of > 8 mEq/L or if not available, a plasma bicarbonate level of < 15 mmol/L or venous plasma lactate > 5 mmol/L. Severe acidosis manifests clinically as respiratory distress (rapid, deep, laboured breathing).
- **Hypoglycaemia:** Blood or plasma glucose < 2.2 mmol/L (< 40 mg/dL)
- **Severe malarial anaemia:** Haemoglobin concentration ≤ 5 g/dL or a haematocrit of ≤ 15% in children ≤ 12 years of age (< 7 g/dL and < 20%, respectively, in adults) with a parasite count > 10 000/μL
- **Renal impairment:** Plasma or serum creatinine > 265 μmol/L (3 mg/dL) or blood urea > 20 mmol/L
- **Jaundice:** Plasma or serum bilirubin > 50 μmol/L (3 mg/dL) with a parasite count > 100 000/μL
- **Pulmonary oedema:** Radiologically confirmed or oxygen saturation < 92% on room air with a respiratory rate > 30/min, often with chest indrawing and crepitations on auscultation
- **Significant bleeding:** Including recurrent or prolonged bleeding from the nose, gums or venepuncture sites; haematemesis or melaena

http://www.who.int/malaria/publications/atoz/9789241549127/en/
Case fatality rate’s

Overall *P.falciparum* (1977-86) 1.3

Range in Europe 0.5 - 5.8

Switzerland 1988-2002 2.2


High antigenic variability
Sporozoites
Ab
Skin → Liver
T cell epitopes are polymorphic
B cell epitopes more or less conserved
Poorly immunogenic; only in circulation for a few minutes
Mosquito
Blood vessel
Lymphatic vessel
Lymph node
Liver
B cell priming or reactivation
CD4
MHC-II
Antigen
RBC
Induction of T cell responses
Gamete
Zygote (oocyste)
Sporozoite
Lower antigenic variability
Blocking of fertilization
Innate immunity
Gut
Salivary gland
Gamete
Sporozoite
Less polymorphic antigens but genetic recombination generates new sporozoite genotypes
Merozoite
Blood vessel
Merozoite
CD8
MHC-I
CD4
MHC-II
Adaptive immunity
Innate immunity
IFN-γ
Oxidation
Merozoite
Phagocytic removal
Prevent infected RBC sequestration
Merozoite
Ab
Opsonization
Variable surface antigens
Vessel wall
RBC
Antigen
NK
CD4
IFN-γ
NO
Phagocytic removal
O2

Riley & Stewart Nat Med 2013;19:168-78
Blood stage

- Trophozoite
- Schizont
- Merozoites
- RBC (ring)
- iRBC
- Circulating mature gametocytes

Mosquito

Drugs to reduce transmission

Merozoites

Delayed development

Sporozoite

Hypnozoites (P. vivax, P. ovale)

Primaquine

Brain

Adherence or sequestration

Cerebral malaria

- Hypoxic brain tissue
- Edema and hemorrhage
- Microvascular obstruction
- Heme
- ROS
- Monocyte
- Apoptotic EC
- Parenchymal and axonal damage

Severe anemia

- Hemolysis
- iRBC
- Impaired erythropoiesis

Metabolic acidosis

- Impaired RBC deformability
- Impaired vasoregulation
- Hypoxia
- Lactic acid
- Vascular leakage
- Inflammation (surrounding tissue)
- EC apoptosis

No drugs

Blood transfusion

No drugs

Fig. 3. Red blood cell deformability: An unparasitized red blood cells shows considerable elongation in order to pass a rigid parasitized red blood cell adhering to the endothelium of the capillary.
Immunity
The development of immunity to malaria

Figure 1. Age-specific parasite rates and positive parasite densities for asexual parasites of *Plasmodium falciparum* and *Plasmodium malariae*. Left axis shows geometric mean positive parasite density as parasites per microliter; right axis shows parasite rate. Category axis shows age groups in days (to age 182 days) and in years. Pregnant women were excluded.
Diagnosis
Malaria: thick blood films

1. Collect a drop of blood from a finger using a capillary tube.

2. Add the drop of blood to a microscope slide.

3. Dry the slide, and it can be sent by mail.
Giemsa stained blood films

If the thick blood film is not distributed on the slide, it will detach under transport or during staining,
*Plasmodium falciparum* – thin blood films

- Ring forms
- Ring forms
- Schizont - early
- Schizont - late
- Schizont - burst
- Schizont - burst
- Gametocyte
- Gametocytes
Sensitivity (parasite threshold) microscopy

COMPARISON OF TWO METHODS FOR ENUMERATING MALARIA PARASITES IN THICK BLOOD FILMS

ESKILD PETERSEN, N. T. MARBIAH, LAURA NEW AND ADAM GOTTSCHAU
Laboratory of Parasitology, and Department of Biostatistics, Statens Seruminstitut, Copenhagen, Denmark; Department of Epidemiology and Population Sciences, London School of Hygiene and Tropical Medicine, London, United Kingdom; European Community Malaria Control Project, Bo, Sierra Leone

Threshold: 2.6 parasites per microliter

(Giemsa stained thick blood films)

Copyright © 1996 by The American Society of Tropical Medicine and Hygiene
Microscopy and limitations of rapid tests

Centers managing patients with malaria must be able to provide round the clock malaria microscopy of thick and thin blood films and parasite density calculations.

Rapid tests may be false negative in cases with very high *P. falciparum* density (Gillet et al. Malaria J 2009; 8:271) (HRP2 deletion in parts of South America).

**Variant *P. ovale*** (Tordrup et al. Malaria J 2011;10:15)

*P. knowlesi* HRP2 based tests will be negative sensitivity variable with other tests

Malaria Rapid Diagnostic. Test Performance
Summary results of WHO product testing of malaria RDTs: rounds 1-6 (2008–2015)

http://www.who.int/malaria/publications/atoz/9789241510035/en/
Performance of Malaria Rapid diagnostic Tests – final assessment

Figure S3: Panel detection score of malaria combination RDTs meeting WHO procurement criteria for false-positive and invalid rates, in phase 2 of rounds 3–6 against wild-type (clinical) samples containing *P. falciparum* and *P. vivax* at low parasite density (200 parasites/μL)

7 NanoSign Malaria Pf/Pan Ag 3.0 RMAP10
8 BIONOTE MALARIA P.f.& P.v. Ag Rapid Test Kit RG19-12
81. Humasis Malaria P.f/P.v Antigen Test AMFV-7025

http://www.who.int/malaria/publications/atoz/9789241510035/en/
Reduction of anti-malarial consumption after rapid diagnostic tests implementation in Dar es Salaam: a before-after and cluster randomized controlled study

Valérie D’Acremont1,2,3*, Judith Kahama-Marongoc,4, Nitonila Swa2,5, Deo Masiwa4, Blaise Genton1,2,5 and Christian Lengeler2,3

Department of Infectious Diseases, The Royal Hospital

© by author
Concentrations of parasite DNA from large volumes of blood gave a consistent analytical detection limit (LOD) of 22 parasites/ml equivalent to 0.022 parasites per μl.
Limitations of RDT’s

Validated only against *P. falciparum* and *P. Vivax*

Capture 2 antigens:

Histidine Rich Protein 2, HRP2, and a “Pan malaria antigen”: LDH or Aldolase

LDH and Aldolase conserved in all 5 human species

But the capture in RDT’s of the pan-malaria antigens not validated
The future? Testing on saliva

Droplet Microfluidics Platform for Highly Sensitive and Quantitative Detection of Malaria-Causing Plasmodium Parasites Based on Enzyme Activity Measurement

Sissel Juul, †, ∗ Christine J. F. Nielsen, †, ∗ Rodrigo Labouriau, †, ∗ Amit Roy, † Cinzia Tesauri, † Pia W. Jensen, ‡ Charlotte Harnsen, † Emil L. Kristoffersen, † Ya-Ling Chiu, † Rikke Frøhlich, † Paola Fiorani, † Janet Cox-Singh, ‡ David Tordrup, † Jørn Koch, † Anne-Lise Bienvenu, † Alessandro Desideri, † Stephane Picot, † Eskild Petersen, ‡ Kam W. Leong, † Yi-Ping Ho, † Magnus Stougaard, †, ∗ and Birgitta R. Knudsen †, ∗

Juul et al ASC Nano 2012
Which method should we use?

Detection levels of different diagnostic methods:

Rapid tests 200 parasites per mm\(^3\)
W.H.O. 2015

Microscopy 2.6 parasites per mm\(^3\)

PCR 0.02 parasites per mm\(^3\)
Imwong et al. JCM 2014
Conclusions

- Microscopy of a Giemsa-stained thick blood film is still the method of choice for the diagnosis of malaria.

- Rapid tests have a slightly lower sensitivity compared to microscopy, but useful where microscopy expertise is not available. Rapid diagnostic test is rapidly taking over from microscopy in tropical Africa.

- PCR is not practical because it is not available outside working hours.
Pregnant women
Malaria in pregnancy

In sub-Saharan Africa, approximately 25 million pregnant women are at risk of *Plasmodium falciparum* infection every year, and one in four women have placental infection at the time of delivery.

Successful prevention reduces the risk of severe maternal anaemia by 38%, low birthweight by 43%, and perinatal mortality by 27% among paucigravidae.

Low birthweight associated with malaria in pregnancy is estimated to result in 100 000 infant deaths in Africa each year.

Malaria and pregnancy

**Treating uncomplicated *P. falciparum* malaria**

Treat children and adults with uncomplicated *P. falciparum* malaria (except pregnant women in their first trimester) with one of the following recommended artemisinin-based combination therapies (ACT):

- artemether + lumefantrine
- artesunate + amodiaquine
- artesunate + mefloquine
- dihydroartemisinin + piperaquine
- artesunate + sulfadoxine–pyrimethamine (SP)

*Strong recommendation, high-quality evidence*

**Duration of ACT treatment**

ACT regimens should provide 3 days' treatment with an artemisinin derivative.

*Strong recommendation, high-quality evidence*

http://www.who.int/malaria/publications/atoz/9789241549127/en/
Treating uncomplicated \textit{P. falciparum} malaria in special risk groups

\textbf{First trimester of pregnancy}

Treat pregnant women with uncomplicated \textit{P. falciparum} malaria during the first trimester with 7 days of quinine + clindamycin.

\textit{Strong recommendation}

\textbf{Patients co-infected with HIV}

In people who have HIV/AIDS and uncomplicated \textit{P. falciparum} malaria, avoid artesunate + SP if they are being treated with co-trimoxazole, and avoid artesunate + amodiaquine if they are being treated with efavirenz or zidovudine.

\textit{Good practice statement}

\url{http://www.who.int/malaria/publications/atoz/9789241549127/en/}
Artemisinin combination therapies:

ACTs used for malaria treatment are artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine and artesunate-sulfadoxine-pyrimethamine.

Many studies have described teratogenic effects of artemisinins in animals as mainly cardiovascular and skeletal defects.

In animal studies the circulating primitive erythroblasts are the main target for embryotoxicity of artemisinin derivatives.

The lack of clinical studies and the results from animal studies led WHO to recommend that ACTs not be used in the first trimester of pregnancy.


Thus, more information is needed to definitively allow artemisinin derivatives use during the whole pregnancy period.
Young adolescent girls are at high risk for adverse pregnancy outcomes in sub-Saharan Africa: an observational multicountry study

The overall prevalence of low birthweight infants and preterm delivery was 10% (371/3851) and 4% (159/3862), respectively. Mothers aged ≤16 years showed higher risk for the delivery of a low birthweight infant (OR: 1.96; 95% CI 1.35 to 2.83). Preterm delivery was associated with young maternal age (≤16 years; OR: 2.62; 95% CI 1.59 to 4.30).

In a subanalysis restricted to primiparous women: preterm delivery, OR 4.28; 95% CI 2.05 to 8.93; low birth weight, OR: 1.29; 95% CI 0.82 to 2.01.
Malaria in pregnancy (MiP) is a major, preventable cause of maternal morbidity and poor birth outcomes. WHO recommends the use of ITNs, effective case management of malaria and anaemia in pregnant women, and in areas of moderate to high malaria transmission in sub-Saharan Africa: intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP).
Intermittent preventive treatment in pregnancy (IPTp)

In malaria-endemic areas in Africa, provide SP-IPTp to all women in their first or second pregnancy as part of antenatal care. Dosing should start in the second trimester and doses should be given at least 1 month apart, with the objective of ensuring that at least three doses are received.

*Strong recommendation, high-quality evidence*

GRADE (see Annex 4, A4.19)
In a systematic review of IPTp, seven trials involving direct comparison of two doses of SP with three or more doses monthly were evaluated. The trials were conducted in Burkina Faso, Kenya, Malawi, Mali and Zambia between 1996 and 2008.

In comparison with two doses of SP, three or more doses:
- Increased the mean birth weight by about 56 g (95% CI, 29–83; seven trials, 2190 participants, *high-quality evidence*);
- Reduced the number of low-birth-weight infants by about 20% (RR, 0.80; 95% CI, 0.69–0.94; seven trials, 2190 participants, *high-quality evidence*);
- Reduced placental parasitaemia by about 50% (RR, 0.51; 95% CI, 0.38–0.68; six trials, 1436 participants, *high-quality evidence*); and
- Reduced maternal parasitaemia by about 33% (RR, 0.68; 95% CI, 0.52–0.89; seven trials, 2096 participants, *high-quality evidence*).
Neonates and children
### TABLE 3 - Pregnancy outcomes based on status of placenta malaria

<table>
<thead>
<tr>
<th>Birth outcome</th>
<th>Placenta malaria</th>
<th>Crude OR (95%CI)</th>
<th>p-value</th>
<th>Adjusted OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 2.5 kg</td>
<td>24</td>
<td>304</td>
<td>2.8 (0.9 – 8.9)</td>
<td>0.08</td>
<td>2.4 (0.7-8.6)</td>
</tr>
<tr>
<td>&lt; 2.5kg</td>
<td>4</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal Hb-level*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 11g/dl</td>
<td>6</td>
<td>104</td>
<td>2.5 (0.9-6.4)</td>
<td>0.061</td>
<td>3.4 (1.3-10.2)</td>
</tr>
<tr>
<td>&lt; 11g/dl</td>
<td>20</td>
<td>140</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA at birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 37 weeks</td>
<td>22</td>
<td>302</td>
<td>4.1 (1.5 – 11.3)</td>
<td>0.006</td>
<td>3.8 (1.3-11.4)</td>
</tr>
<tr>
<td>&lt;37 weeks</td>
<td>6</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: GA = Gestational age; OR = odds ratio; CI = confidence interval; Hb = Haemoglobin level
*There were 80 participants with no Hb results

Immune priming ? Intra-uterine malaria exposure

Revised dose recommendation for dihydroartemisinin + piperquine in young children

Children < 25kg treated with dihydroartemisinin + piperquine should receive a minimum of 2.5 mg/kg body weight (bw) per day of dihydroartemisinin and 20 mg/kg bw per day of piperquine daily for 3 days.

Strong recommendation based on pharmacokinetic modelling

Infants less than 5kg body weight

Treat infants weighing < 5 kg with uncomplicated *P. falciparum* malaria with ACT at the same mg/kg bw target dose as for children weighing 5 kg.

Strong recommendation

http://www.who.int/malaria/publications/atoz/9789241549127/en/
Infants less than 6 months of age

Both congenital and neonatal malaria infections are considered to be very rare. (Covell, 1950; Bruce-Chwatt, 1952).

Though existing data on prevalence and burden of disease in young infants are still limited and often contradictory (Mwaniki et al. 2010), it appears that while sub-clinical asymptomatic infection is not uncommon, clinical disease due to Pf infection is quite rare in infants under 5 months of age.

(Sehgal et al. 1989; McGuinness et al. 1998; Snow et al. 1998; Franks et al. 2001).
Iron supplements to anemic children?

Iron supplement to infants may lead to increase prevalence of malaria and pneumonia


Iron supplements in older children improve hemoglobin levels but also in increased incidence of clinical malaria


Folate supplements had no influence on the hemoglobin levels of children with Malaria in The Gambia in a randomised, controlled study

11.3 | SEASONAL MALARIA CHEMOPREVENTION WITH AMODIAQUINE + SULFADOXINE – PYRIMETHAMINE

Seasonal malaria chemoprophylaxis

In areas with highly seasonal malaria transmission in the sub-Saharan region of Africa, provide SMC with monthly amodiaquine + SP for all children < 6 years during each transmission season.

*Strong recommendation, high-quality evidence*
Figure 17. Global age-sex distribution of malaria incidence (A) and deaths (B) in 2013
A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants.

Four-year efficacy of RTS,S/AS01E and its interaction with malaria exposure

Kilifi - Kenya

Thank you

Wadi Tiwi
New Malaria Test, illumigene® Malaria, Sets a New Gold Standard for Diagnosis

Meridian Bioscience, Inc., Cincinnati, Ohio (NASDAQ: VIVO) today announced that it has received the CE Mark for illumigene® Malaria, a novel, highly accurate test developed by Meridian with the technical assistance of the Centers for Disease Control and Prevention (CDC) and Cheikh Anta Diop University of Dakar, Senegal. The test is up to 80,000 times more sensitive at detecting the malaria parasite than conventional tests, potentially revolutionizing malaria diagnosis and establishing a new gold standard. Using innovative molecular LAMP technology, illumigene results are available in under one hour, and the test is easy to use as it does not rely on high level technical expertise. This is a major step forward for people with malaria as faster, more accurate diagnoses.
Revolutionize your lab’s diagnostic accuracy with the molecular performance of illumigene® Malaria.

- Sensitivity — Analytical sensitivity up to 80,000x more than conventional methods\(^1\)^ \(^2\)
- Speed — Precise results in less than one hour
- Simplicity — Minimal technical expertise required
- Affordable — No capital investment necessary

100 parasites necessary per microliter to detect malaria by using illumigene®

ONLY 0.06-2

500-5,000 parasites necessary per microliter to detect malaria by using illumigene®
Spatial limits of *Plasmodium vivax* in 2005

Several sources of information on malaria risk (notably international travel health guidelines on malaria chemoprophylaxis, altitude limits for dominant vectors, climate limits for malaria transmission and human population density thresholds) have been combined in a GIS to generate this map. See Guerra et al (2006) Advances in Parasitology, 62: 157-179 and Guerra et al (2006) Trends in Parasitology, 22: 353-358 for details of the methodology. This map is provided only as an interim guide. The Malaria Atlas Project (www.map.ox.ac.uk) is funded, largely by the Wellcome Trust, UK, to assemble the medical intelligence required to improve future iterations of these limits.
Figure 16. Global malaria incidence (A) and deaths (B), 1990–2013, for all ages and both sexes combined.
There were 13 miscarriages (1 miscarriage in the artemether–lumefantrine group and 4 in each of the other three groups).

There were 78 stillbirths overall, with 16 stillbirths occurring in 856 births (1.9%) in the artemether–lumefantrine group, 17 in 815 (2.1%) in the amodiaquine–artesunate group, 22 in 818 (2.7%) in the dihydroartemisinin–piperaquine group, and 23 in 821 (2.8%) in the mefloquine–artesunate group.

The proportion of live births did not differ significantly among the treatment groups (p=0.85).

In conclusion, artemether–lumefantrine was associated with the fewest adverse effects and with acceptable cure rates but provided the shortest post-treatment prophylaxis. Dihydroartemisinin–piperaquine had the best efficacy and an acceptable safety profile.
Primaquine kills gametocytes and thus inhibit transmission.

Gametocytes can be found up to 3 weeks after treatment and is not inhibited by the other anti-malarials

http://www.who.int/malaria/publications/atoz/9789241549127/en/
Mefloquine versus Sulfadoxine–Pyrimethamine for Intermittent Preventive Treatment in Pregnancy: A Joint Analysis on Efficacy and Tolerability

Valérie Briand,* Sylvie Escolano, Valérie Journot, Achille Massougbdji, Michel Cot, and Pascale Tubert-Bitter

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Missing data (n)</th>
<th>Prevalence in MQ group (%)</th>
<th>Prevalence in SP group (%)</th>
<th>Univariate comparison P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBW†</td>
<td>136</td>
<td>8.0</td>
<td>9.8</td>
<td>0.22</td>
</tr>
<tr>
<td>LBW‡</td>
<td>88</td>
<td>9.7</td>
<td>11.1</td>
<td>0.36</td>
</tr>
<tr>
<td>Placental malaria</td>
<td>282</td>
<td>1.7</td>
<td>4.4</td>
<td>0.004</td>
</tr>
<tr>
<td>Anemia</td>
<td>335</td>
<td>16.5</td>
<td>20.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Low tolerability§</td>
<td>7</td>
<td>7.1</td>
<td>3.7</td>
<td>0.002</td>
</tr>
</tbody>
</table>

LBW = low birth weight; IPTp = intermittent preventive treatment; MQ = mefloquine; SP = sulfadoxine–pyrimethamine.