

# LIST OF ANNEXES

Annex 1.	The guidelines development process	77
Annex 2.	Adaptation of <i>WHO malaria treatment guidelines</i> for use in countries	83
Annex 3.	Pharmacology of antimalarial drugs	87
Annex 4.	Antimalarials and malaria transmission	133
Annex 5.	Malaria diagnosis	147
Annex 6.	Resistance to antimalarials	155
Annex 7.	Uncomplicated <i>P. falciparum</i> malaria	185
Annex 8.	Malaria treatment and HIV/AIDS	199
Annex 9.	Treatment of severe <i>P. falciparum</i> malaria	207
Annex 10.	Treatment of <i>P. vivax</i> , <i>P. ovale</i> and <i>P. malariae</i> infections	225

# ANNEX 1

## THE GUIDELINES DEVELOPMENT PROCESS

### A1.1 Treatment recommendations

The *WHO Guidelines for the Treatment of Malaria* were developed in accordance with the guidance established by WHO for the formulation of such guidelines.<sup>21</sup>

The development process was undertaken at a technical consultation on malaria treatment guidelines and by the Technical Guidelines Development Group, chaired by Professor Nick White (participants are listed below). Conflict of interest statements were received from all participants, and no conflict of interest was declared by any of the participants. The first technical consultation was convened in April 2004, at which the target audience for the guidelines was defined and the scope of the guidelines and key questions to be addressed were determined.

Following the first meeting, contracts for systematic search and reviews of relevant evidence were awarded to research groups from two institutions: the Liverpool School of Tropical Medicine, Liverpool, England and Mahidol University, Bangkok, Thailand. The search strategies employed included a search of the following databases:

- the Cochrane Infectious Diseases Group trials register (up to June 2004),\*
- the Cochrane Central Register of Controlled Trials (CENTRAL) published in The Cochrane Library (Issue 2, 2004),\*
- MEDLINE (1966 to June 2004),
- EMBASE (1980 to June 2004),
- LILACS (1982 to June 2004).

The evidence for RCTs was assembled in collaboration with Clinical Evidence, a product of BMJ Knowledge.

The following search terms were used:

- malaria (free text),
- malaria (controlled vocabulary, MESH or EMTREE).

The terms were used in combination with the search strategy for retrieving randomized and controlled clinical trials developed by The Cochrane Collaboration. Key words relative to currently available antimalarial drugs were used for

<sup>21</sup> *Guidelines for guidelines*. Geneva, World Health Organization, 2003 (document EIP/GPE/EQC/2003.1).

each section of the review. Where indicated, specific authors and research groups were contacted for more information on published work and work in progress.

In formulating recommendations, evidence was graded in order of priority as follows:

- formal systematic reviews, such as Cochrane reviews, including more than one randomized control trial,
- comparative trials without formal systematic review,
- observational studies (e.g. surveillance, pharmacological data),
- expert opinion/consensus.

The Technical Guidelines Development Group held its first meeting in August 2004 and produced a preliminary draft that was presented and revised at the second technical consultation in October 2004. The revised draft of the guidelines was sent out for external peer review in November 2004. At a subsequent meeting of the Technical Guidelines Development Group in January 2005, the document was revised further in the light of comments received. The Guidelines development process and publication was fully funded by WHO. It is planned that the evidence will be reviewed on an annual basis, and that the guidelines will be updated periodically. Similarly a mechanism for periodic monitoring and evaluating the use of the treatment guidelines in countries would be established.

## **A1.2 Members of the Technical Guidelines Development Group**

Dr D. Baza, National Malaria Control Programme Manager, Ministry of Health, Burundi

Dr D. Bethell\*, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Professor A. Bjorkman, Division of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden

Professor M. Boulos, Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Professor M. A. Faiz, Department of Medicine, Dhaka Medical College, Bangladesh

- Professor P. Garner\*, Liverpool School of Tropical Medicine,  
Liverpool, England
- Professor O. Gaye, Service de Parasitologie, Faculté de Médecine,  
Université Cheikh Anta Diop, Dakar-Fann, Senegal
- Dr T. Ghebremeskel, National Malaria Programme Manager, Ministry  
of Health, Asmara, Eritrea
- Dr S. Hill\*, Discipline of Clinical Pharmacology, Faculty of Medicine &  
Health Sciences, University of Newcastle, Newcastle Mater Hospital,  
Newcastle, Australia
- Dr K. Jones\*, Clinical Research Fellow, Liverpool School of Tropical  
Medicine, Liverpool, England
- Dr S. Lutalo, Consultant Physician, Harare Central Hospital,  
Harare, Zimbabwe
- Dr A. McCarthy, Director, Tropical Medicine and International Division of  
Infectious Diseases, Ottawa Hospital General Campus, Ottawa, Canada
- Dr O. Mokuolu\*, Consultant Paediatrician, University of Ilorin Teaching  
Hospital, Ilorin, Nigeria
- Dr S. Nyirenda, Consultant Physician, Department of Medicine,  
University Teaching Hospital, Lusaka, Zambia
- Dr E. Tjitra\*, Senior Researcher, National Institute of Health  
& Development, Ministry of Health, Jakarta, Indonesia
- Dr L.S. Vestergaard, Laboratory of Parasitic Diseases, Statens Serum  
Institut, and Department of International Health, University of  
Copenhagen, Copenhagen, Denmark
- Professor N. White\* (*Chairman*) Faculty of Tropical Medicine,  
Mahidol University, Bangkok, Thailand
- 
- Dr D. Bell, Malaria and Parasitic Diseases, WHO Regional Office for  
the Western Pacific, Manila, Philippines
- Dr A. Bosman, Roll Back Malaria Department, World Health Organization,  
Geneva, Switzerland
- Dr C. Delacollette, Roll Back Malaria Department, World Health  
Organization, Geneva, Switzerland
- Dr M. Gomes, UNDP/UNICEF/World Bank/WHO Special Programme for  
Research and Training in Tropical Diseases, World Health Organization,  
Geneva, Switzerland

- Dr R. Gray, Essential Drugs and Medicine, World Health Organization, Geneva, Switzerland
- Dr K.N. Mendis\*, Roll Back Malaria Department, World Health Organization, Geneva, Switzerland
- Dr F. Nafu-Traoré, Roll Back Malaria Department, World Health Organization, Geneva, Switzerland
- Dr B.L. Nahlen, Roll Back Malaria Department, World Health Organization, Geneva, Switzerland
- Dr P.E. Olumese\* (*Secretary*), Roll Back Malaria Department, World Health Organization, Geneva, Switzerland
- Dr C. Ondari\*, Essential Drugs and Medicine, World Health Organization, Geneva, Switzerland
- Dr R. Ridley\*, UNDP/UNICEF/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, World Health Organization, Geneva, Switzerland
- Dr A.E.C. Rietveld\*, Roll Back Malaria Department, World Health Organization, Geneva, Switzerland
- Dr P. Ringwald, Roll Back Malaria Department, World Health Organization, Geneva, Switzerland
- Dr I. Sanou, Malaria, WHO Regional Office for Africa, Harare, Zimbabwe.
- Dr A. Schapira, Roll Back Malaria Department, World Health Organization, Geneva, Switzerland
- Dr T. Sukwa\*, Malaria, WHO Regional Office for Africa, Harare, Zimbabwe
- Dr T. Tantorres, Evidence and Information for Policy, World Health Organization, Geneva, Switzerland
- Dr W. Taylor, UNDP/UNICEF/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, World Health Organization, Geneva, Switzerland
- Dr Y. Touré, UNDP/UNICEF/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, World Health Organization, Geneva, Switzerland
- Dr W.M. Were, Roll Back Malaria Department, World Health Organization, Geneva, Switzerland

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\* Members of the Guidelines drafting committee.

## ANNEX 2

# ADAPTATION OF *WHO MALARIA TREATMENT GUIDELINES* FOR USE IN COUNTRIES

### A2.1 Background

WHO has recently convened technical consultations aimed at developing guidelines for the treatment of malaria.<sup>22</sup>

The guidelines are generic in nature and should therefore be adapted by regions and countries alike to take account of local conditions, especially when formulating implementation and scale-up strategies.

A number of malaria endemic countries have not yet elaborated national malaria treatment guidelines, although treatment protocols may be available at the health-care provider level. Furthermore, existing national guidelines need to be updated as many countries, especially in sub-Saharan Africa, are adopting and starting to implement policies specifying the use of artemisinin combination therapies (ACTs).

This annex provides orientation and guidance on the process countries should follow in adapting the content of the generic malaria treatment guidelines provided in the main document.

### A2.2 The development process

The ministry of health should take the lead in the process of developing national malaria treatment guidelines.<sup>23</sup>

The proposed steps include the following.

- *A national workshop on the malaria treatment guidelines* is the first step at country level. This workshop will review any current national malaria treatment guidelines, identify specific issues that need to be addressed and provide major policy recommendations.
- *Drafting/updating the national malaria treatment guidelines*. Following the national workshop, the national malaria case management committee (or its equivalent) should spearhead the development of new national malaria treatment guidelines in accordance with the standard outline set out below.

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<sup>22</sup> The guidelines development process is described in Annex 1.

<sup>23</sup> The preparation of the treatment guidelines is only one component of implementation of antimalarial treatment policy.

- *A consensus workshop on the national malaria treatment guidelines* should then be arranged to present, discuss and adopt the draft national malaria treatment guidelines.
- *Finalization and dissemination.* The national malaria treatment guidelines are finalized, officially endorsed and disseminated.

### A2.3 The content

It is recommended that national malaria treatment guidelines are presented in a similar way as WHO Guidelines on the Treatment of Malaria. The following outline is suggested:

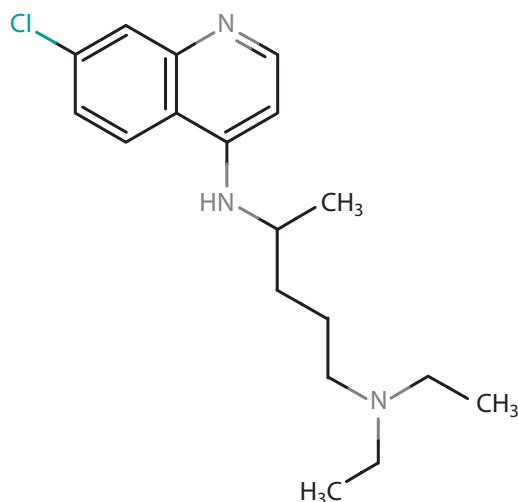
1. General introduction
  - Epidemiological situation and parasite distribution
  - National drug resistance pattern
2. Diagnosis of malaria
  - Clinical diagnosis
  - Role of parasitological diagnosis
3. Treatment of *P. falciparum* malaria or the most prevalent species in the country
  - Uncomplicated malaria
    - definition
    - treatment objectives
    - treatment recommendations
    - treatment in specific populations and situations
  - Severe malaria
    - definition
    - treatment objectives
    - treatment recommendations
    - pre-referral treatment options
    - management in epidemic situations
4. Treatment of malaria caused by other species
5. Disease management at the different levels of the health care delivery system
6. Annexes – Relevant annexes should be attached to provide more detailed information on, for example, dosages of drugs, specific data on therapeutic efficacy of antimalarial medicines in the country, other available evidence for treatment recommendation, etc.

## ANNEX 3

### PHARMACOLOGY OF ANTIMALARIAL DRUGS

#### A3.1 Chloroquine

Molecular weight: 436.0



Chloroquine is a 4-aminoquinoline that has been used extensively for the treatment and prevention of malaria. Widespread resistance has now rendered it virtually useless against *P. falciparum* infections in most parts of the world, although it still maintains considerable efficacy for the treatment of *P. vivax*, *P. ovale* and *P. malariae* infections. As with other 4-aminoquinolines, it does not produce radical cure.

Chloroquine interferes with parasite haem detoxification (1, 2). Resistance is related to genetic changes in transporters (PfCRT, PfMDR), which reduce the concentrations of chloroquine at its site of action, the parasite food vacuole.

#### Formulations

- Tablets containing 100 mg or 150 mg of chloroquine base as hydrochloride, phosphate or sulfate.

#### Pharmacokinetics

Chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract when taken orally, although peak plasma concentrations can vary considerably. Absorption is also very rapid following intramuscular and subcutaneous

administration (3–5). Chloroquine is extensively distributed into body tissues, including the placenta and breast milk, and has an enormous total apparent volume of distribution. The relatively small volume of distribution of the central compartment means that transiently cardiotoxic levels may occur following intravenous administration unless the rate of parenteral delivery is strictly controlled. Some 60% of chloroquine is bound to plasma proteins, and the drug is eliminated slowly from the body via the kidneys, with an estimated terminal elimination half-life of 1–2 months. Chloroquine is metabolized in the liver, mainly to monodesethylchloroquine, which has similar activity against *P. falciparum*.

### Toxicity

Chloroquine has a low safety margin and is very dangerous in overdose. Larger doses of chloroquine are used for the treatment of rheumatoid arthritis than for malaria, so adverse effects are seen more frequently in patients with arthritis. The drug is generally well tolerated. The principle limiting adverse effects in practice are the unpleasant taste, which may upset children, and pruritus, which may be severe in dark-skinned patients (6). Other less common side effects include headache, various skin eruptions and gastrointestinal disturbances, such as nausea, vomiting and diarrhoea. More rarely central nervous system toxicity including, convulsions and mental changes may occur. Chronic use (>5 years continuous use as prophylaxis) may lead to eye disorders, including keratopathy and retinopathy. Other uncommon effects include myopathy, reduced hearing, photosensitivity and loss of hair. Blood disorders, such as aplastic anaemia, are extremely uncommon (7).

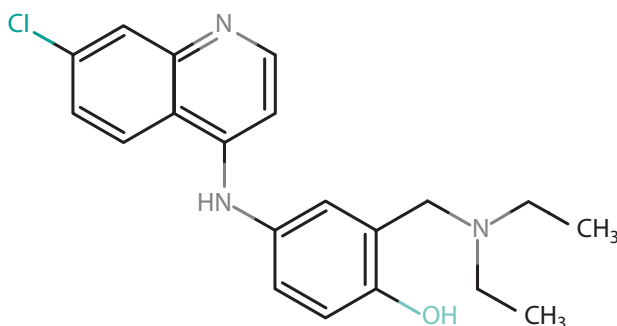
Acute overdose is extremely dangerous and death can occur within a few hours. The patient may progress from feeling dizzy and drowsy with headache and gastrointestinal upset, to developing sudden visual disturbance, convulsions, hypokalaemia, hypotension and cardiac arrhythmias. There is no specific treatment, although diazepam and epinephrine (adrenaline) administered together are beneficial (8, 9).

### Drug interactions

Major interactions are very unusual. There is a theoretical increased risk of arrhythmias when chloroquine is given with halofantrine or other drugs that prolong the electrocardiograph QT interval; a possible increased risk of convulsions with mefloquine; reduced absorption with antacids; reduced metabolism and clearance with cimetidine; an increased risk of acute dystonic reactions with metronidazole; reduced bioavailability of ampicillin and praziquantel; reduced therapeutic effect of thyroxine; a possible antagonistic effect on the antiepileptic effects of carbamazepine and sodium valproate; and increased plasma concentrations of cyclosporine.

## A3.2 Amodiaquine

Molecular weight: 355.9



Amodiaquine is a Mannich base 4-aminoquinoline with a mode of action similar to that of chloroquine. It is effective against some chloroquine-resistant strains of *P. falciparum*, although there is cross-resistance.

### Formulations

- Tablets containing 200 mg of amodiaquine base as hydrochloride or 153.1 mg of base as chlorohydrate.

### Pharmacokinetics

Amodiaquine hydrochloride is readily absorbed from the gastrointestinal tract. It is rapidly converted in the liver to the active metabolite desethylamodiaquine, which contributes nearly all of the antimalarial effect (10). There are insufficient data on the terminal plasma elimination half-life of desethylamodiaquine. Both amodiaquine and desethylamodiaquine have been detected in the urine several months after administration.

### Toxicity

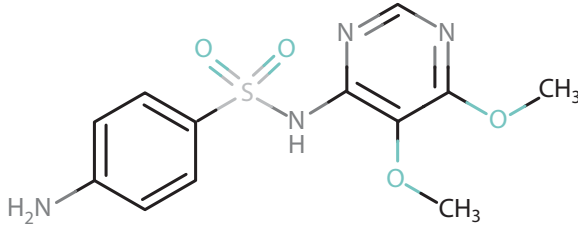
The adverse effects of amodiaquine are similar to those of chloroquine. Amodiaquine is associated with less pruritus and is more palatable than chloroquine, but is associated with a much higher risk of agranulocytosis and, to a lesser degree, of hepatitis when used for prophylaxis (11). The risk of a serious adverse reaction with prophylactic use (which is no longer recommended) appears to be between 1 in 1000 and 1 in 5000. It is not clear whether the risks are lower when amodiaquine is used to treat malaria. Following overdose cardiotoxicity appears to be less frequent than with chloroquine. Large doses of amodiaquine have been reported to cause syncope, spasticity, convulsions and involuntary movements.

### Drug interactions

There are insufficient data.

### A3.3 Sulfadoxine

Molecular weight: 310.3



Sulfadoxine is a slowly eliminated sulfonamide. It is very slightly soluble in water. Sulfonamides are structural analogues and competitive antagonists of *p*-aminobenzoic acid. They are competitive inhibitors of dihydropteroate synthase, the bacterial enzyme responsible for the incorporation of *p*-aminobenzoic acid in the synthesis of folic acid.

#### Formulations

Sulfadoxine is used in a fixed-dose combination of 20 parts sulfadoxine with 1 part pyrimethamine and may be administered orally or by the intramuscular route:

- Tablets containing 500 mg of sulfadoxine and 25 mg of pyrimethamine.
- Ampoules containing 500 mg of sulfadoxine and 25 mg of pyrimethamine in 2.5 ml of injectable solution for intramuscular use.

#### Pharmacokinetics

Sulfadoxine is readily absorbed from the gastrointestinal tract. Peak blood concentrations occur about 4 h after an oral dose. The terminal elimination half-life is 4–9 days. Around 90–95% is bound to plasma proteins. It is widely distributed to body tissues and fluids, passes into the fetal circulation and is detectable in breast milk. The drug is excreted in urine, primarily unchanged.

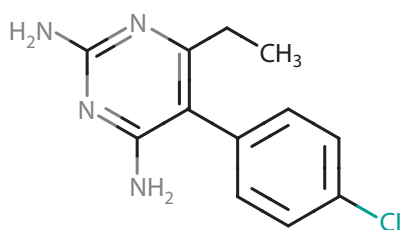
#### Toxicity

Sulfadoxine shares the adverse effect profile of other sulfonamides, although allergic reactions can be severe because of its slow elimination. Nausea, vomiting, anorexia and diarrhoea may occur. Crystalluria causing lumbar pain, haematuria and oliguria is rare compared with more rapidly eliminated sulphonamides. Hypersensitivity reactions may affect different organ system. Cutaneous manifestations can be severe and include pruritus, photosensitivity

reactions, exfoliative dermatitis, erythema nodosum, toxic epidermal necrolysis and Stevens-Johnson syndrome (12). Treatment with sulfadoxine should be stopped in any patient developing a rash because of the risk of severe allergic reactions (13). Hypersensitivity to sulfadoxine may also cause interstitial nephritis, lumbar pain, haematuria and oliguria. This is due to crystal formation in the urine (crystalluria) and may be avoided by keeping the patient well hydrated to maintain a high urine output. Alkalinization of the urine will also make the crystals more soluble. Blood disorders that have been reported include agranulocytosis, aplastic anaemia, thrombocytopenia, leukopenia and hypoproteinaemia. Acute haemolytic anaemia is a rare complication, which may be antibody mediated or associated with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Other adverse effects, which may be manifestations of a generalized hypersensitivity reaction include fever, interstitial nephritis, a syndrome resembling serum sickness, hepatitis, myocarditis, pulmonary eosinophilia, fibrosing alveolitis, peripheral neuropathy and systemic vasculitis, including polyarteritis nodosa. Anaphylaxis has been reported only rarely. Other adverse reactions that have been reported include hypoglycaemia, jaundice in neonates, aseptic meningitis, drowsiness, fatigue, headache, ataxia, dizziness, convulsions, neuropathies, psychosis and pseudomembranous colitis.

### A3.4 Pyrimethamine

Molecular weight: 248.7



Pyrimethamine is a diaminopyrimidine used in combination with a sulfonamide, usually sulfadoxine or dapsone. It exerts its antimalarial activity by inhibiting plasmodial dihydrofolate reductase thus indirectly blocking the synthesis of nucleic acids in the malaria parasite. It is a slow-acting blood schizonticide and is also possibly active against pre-erythrocytic forms of the malaria parasite and inhibits sporozoite development in the mosquito vector. It is effective against all four human malarias, although resistance has emerged

rapidly. Pyrimethamine is also used in the treatment of toxoplasmosis, and isosporiasis and as prophylaxis against *Pneumocystis carinii* pneumonia. Pyrimethamine is no longer used alone as an antimalarial, only in synergistic combination with slowly eliminated sulfonamides for treatment (sulfadoxine, sulfalene) or with dapsone for prophylaxis.

### Formulations

Pyrimethamine is currently used mainly in a fixed-dose combination with slowly eliminated sulfonamides, either of 20 parts sulfadoxine with 1 part pyrimethamine for which there are oral and parenteral formulations:

- Tablets containing 500 mg of sulfadoxine and 25 mg of pyrimethamine.
- Ampoules containing 500 mg of sulfadoxine and 25 mg of pyrimethamine in 2.5 ml of injectable solution for intramuscular use.

### Pharmacokinetics

Pyrimethamine is almost completely absorbed from the gastrointestinal tract and peak plasma concentrations occur 2–6 h after an oral dose. It is mainly concentrated in the kidneys, lungs, liver and spleen, and about 80–90% is bound to plasma proteins. It is metabolized in the liver and slowly excreted via the kidneys. The plasma half-life is around 4 days. Pyrimethamine crosses the blood-brain barrier and the placenta and is detectable in breast milk. Absorption of the intramuscular preparation is incomplete and insufficiently reliable for this formulation to be recommended (14).

### Toxicity

Pyrimethamine is generally very well tolerated. Administration for prolonged periods may cause depression of haematopoiesis due to interference with folic acid metabolism. Skin rashes and hypersensitivity reactions also occur. Larger doses may cause gastrointestinal symptoms such as atrophic glossitis, abdominal pain and vomiting, haematological effects including megaloblastic anaemia, leukopenia, thrombocytopenia and pancytopenia, and central nervous system effects such as headache and dizziness.

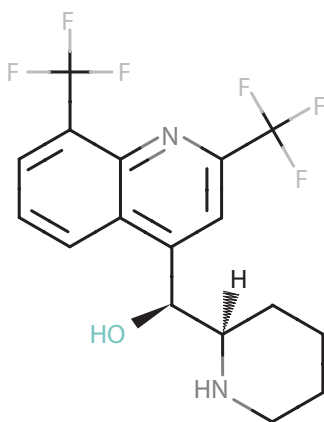
Acute overdosage of pyrimethamine can cause gastrointestinal effects and stimulation of the central nervous system with vomiting, excitability and convulsions. Tachycardia, respiratory depression, circulatory collapse and death may follow. Treatment of overdosage is supportive.

### Drug interactions

Administration of pyrimethamine with other folate antagonists such as cotrimoxazole, trimethoprim, methotrexate or with phenytoin may exacerbate bone marrow depression. Given with some benzodiazepines, there is a risk of hepatotoxicity.

## A3.5 Mefloquine

Molecular weight: 378.3



Mefloquine is a 4-methanolquinoline and is related to quinine. It is soluble in alcohol but only very slightly soluble in water. It should be protected from light. The drug is effective against all forms of malaria.

### Formulations

Mefloquine is administered by mouth as the hydrochloride salt (250 mg base equivalent to 274 mg hydrochloride salt):

- Tablets containing either 250 mg salt (United States of America) or 250 mg base (elsewhere).

### Pharmacokinetics

Mefloquine is reasonably well absorbed from the gastrointestinal tract but there is marked interindividual variation in the time required to achieve peak plasma concentrations. Splitting the 25 mg/kg dose into two parts given at an interval of 6–24 h augments absorption and improves tolerability (15). Mefloquine undergoes enterohepatic recycling. It is approximately 98% bound to plasma proteins and is widely distributed throughout the body. The pharmacokinetics

of mefloquine may be altered by malaria infection with reduced absorption and accelerated clearance (16, 17). When administered with artesunate, blood concentrations are increased, probably as an indirect effect of increased absorption resulting from more rapid resolution of symptoms (15). Mefloquine is excreted in small amounts in breast milk. It has a long elimination half-life of around 21 days, which is shortened in malaria to about 14 days, possibly because of interrupted enterohepatic cycling (18–20). Mefloquine is metabolized in the liver and excreted mainly in the bile and faeces. Its pharmacokinetics show enantioselectivity after administration of the racemic mixture, with higher peak plasma concentrations and area under the curve values, and lower volume of distribution and total clearance of the SR enantiomer than its RS antipode (21–23).

### Toxicity

Minor adverse effects are common following mefloquine treatment, most frequently nausea, vomiting, abdominal pain, anorexia, diarrhoea, headache, dizziness, loss of balance, dysphoria, somnolence and sleep disorders, notably insomnia and abnormal dreams. Neuropsychiatric disturbances (seizures, encephalopathy, psychosis) occur in approximately 1 in 10 000 travellers receiving mefloquine prophylaxis, 1 in 1000 patients treated in Asia, 1 in 200 patients treated in Africa, and 1 in 20 patients following severe malaria (24–27). Other side effects reported rarely include skin rashes, pruritus and urticaria, hair loss, muscle weakness, liver function disturbances and very rarely thrombocytopenia and leukopenia. Cardiovascular effects have included postural hypotension, bradycardia and, rarely, hypertension, tachycardia or palpitations and minor changes in the electrocardiogram. Fatalities have not been reported following overdosage, although cardiac, hepatic and neurological symptoms may be seen. Mefloquine should not be given with halofantrine because it exacerbates QT prolongation. There is no evidence of an adverse interaction with quinine (28).

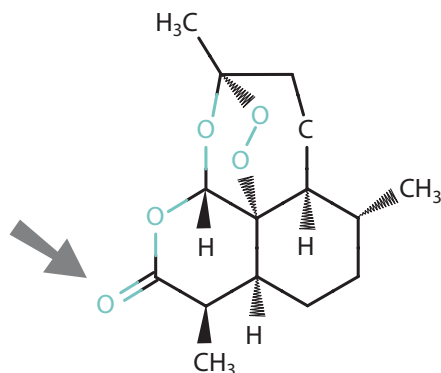
### Drug interactions

There is a possible increase in the risk of arrhythmias if mefloquine is given together with beta blockers, calcium channel blockers, amiodarone, pimozide, digoxin or antidepressants; there is also a possible increase in the risk of convulsions with chloroquine and quinine. Mefloquine concentrations are increased when given with ampicillin, tetracycline and metoclopramide. Caution should be observed with alcohol.

## A3.6 Artemisinin and its derivatives

### A3.6.1 Artemisinin

Molecular weight: 282.3



Artemisinin, also known as qinghaosu, is a sesquiterpene lactone extracted from the leaves of *Artemisia annua* (sweet wormwood). It has been used in China for the treatment of fever for over a thousand years. It is a potent and rapidly acting blood schizontocide and is active against all *Plasmodium* species. It has an unusually broad activity against asexual parasites, killing all stages from young rings to schizonts. In *P. falciparum* malaria, artemisinin also kills the gametocytes – including the stage 4 gametocytes, which are otherwise sensitive only to primaquine. Artemisinin and its derivatives inhibit an essential calcium adenosine triphosphatase, PfATPase 6 (29). Artemisinin has now largely given way to the more potent dihydroartemisinin and its derivatives, artemether, artemotil and artesunate. The three latter derivatives are converted back *in vivo* to dihydroartemisinin. These drugs should be given as combination therapy to protect them from resistance.

#### Formulations

A wide variety of formulations for oral, parenteral and rectal use are available. These include:

- Tablets and capsules containing 250 mg of artemisinin.
- Suppositories containing 100 mg, 200 mg, 300 mg, 400 mg or 500 mg of artemisinin.

#### Pharmacokinetics

Peak plasma concentrations occur around 3 h and 11 h following oral and rectal administration respectively (30). Artemisinin is converted to inactive metabolites via the cytochrome P450 enzyme CYP2B6 and other enzymes.

Artemisinin is a potent inducer of its own metabolism. The elimination half-life is approximately 1 h (31).

### Toxicity

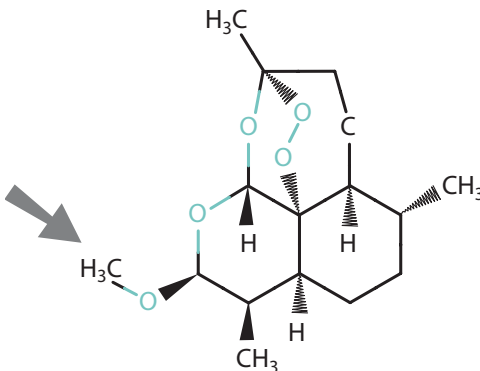
Artemisinin and its derivatives are safe and remarkably well tolerated (32, 33). There have been reports of mild gastrointestinal disturbances, dizziness, tinnitus, reticulocytopenia, neutropenia, elevated liver enzyme values, and electrocardiographic abnormalities, including bradycardia and prolongation of the QT interval, although most studies have not found any electrocardiographic abnormalities. The only potentially serious adverse effect reported with this class of drugs is type 1 hypersensitivity reactions in approximately 1 in 3000 patients (34). Neurotoxicity has been reported in animal studies, particularly with very high doses of intramuscular artemotil and artemether, but has not been substantiated in humans (35–37). Similarly, evidence of death of embryos and morphological abnormalities in early pregnancy have been demonstrated in animal studies (37a). Artemisinin has not been evaluated in the first trimester of pregnancy so should be avoided in first trimester patients with uncomplicated malaria until more information is available.

### Drug interactions

None known.

### A3.6.2 Artemether

Molecular weight: 298.4



Artemether is the methyl ether of dihydroartemisinin. It is more lipid soluble than artemisinin or artesunate. It can be given as an oil-based intramuscular injection or orally. It is also coformulated with lumefantrine (previously referred to as benflumetol) for combination therapy.

### Formulations

- Capsules containing 40 mg of artemether.
- Tablets containing 50 mg of artemether.
- Ampoules of injectable solution for intramuscular injection containing 80 mg of artemether in 1 ml for adults or 40 mg of artemether in 1 ml for paediatric use.

In a coformulation with lumefantrine:

- Tablets containing 20 mg of artemether and 120 mg of lumefantrine.

### Pharmacokinetics

Peak plasma concentrations occur around 2–3 h after oral administration (38). Following intramuscular injection, absorption is very variable, especially in children with poor peripheral perfusion: peak plasma concentrations generally occur after around 6 h but absorption is slow and erratic and times to peak can be 18 h or longer in some cases (39–41). Artemether is metabolized to dihydroartemisinin, the active metabolite. After intramuscular administration, artemether predominates, whereas after oral administration dihydroartemisinin predominates. Biotransformation is mediated via the cytochrome P<sub>450</sub> enzyme CYP3A<sub>4</sub>. Autoinduction of metabolism is less than with artemisinin. Artemether is 95% bound to plasma proteins. The elimination half-life is approximately 1 h, but following intramuscular administration the elimination phase is prolonged because of continued absorption. No dose modifications are necessary in renal or hepatic impairment.

### Toxicity

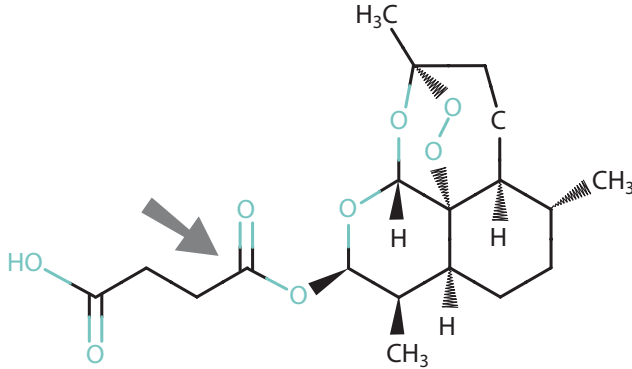
In all species of animals tested, intramuscular artemether and artemotil cause an unusual selective pattern of neuronal damage to certain brain stem nuclei. Neurotoxicity in experimental animals is related to the sustained blood concentrations that follow intramuscular administration (42), since it is much less frequent when the same doses are given orally, or with similar doses of water-soluble drugs such as artesunate. Clinical, neurophysiological and pathological studies in humans have not shown similar findings with therapeutic use of these compounds (40). Toxicity is otherwise similar to that of artemisinin.

### Drug interactions

None known.

### A3.6.3 Artesunate

Molecular weight: 384.4



Artesunate is the sodium salt of the hemisuccinate ester of artemisinin. It is soluble in water but has poor stability in aqueous solutions at neutral or acid pH. In the injectable form, artesunic acid is drawn up in sodium bicarbonate to form sodium artesunate immediately before injection. Artesunate can be given orally, rectally or by the intramuscular or intravenous routes. There are no coformulations currently available.

#### Formulations

- Tablets containing 50 mg or 200 mg of sodium artesunate.
- Ampoules: intramuscular or intravenous injection containing 60 mg of anhydrous artesunic acid with a separate ampoule of 5% sodium bicarbonate solution.
- Rectal capsules containing 100 mg or 400 mg of sodium artesunate.

#### Pharmacokinetics

Artesunate is rapidly absorbed, with peak plasma levels occurring 1.5 h and 2 h and 0.5 h after oral, rectal and intramuscular administration, respectively (43–47). It is almost entirely converted to dihydroartemisinin, the active metabolite (30). Elimination of artesunate is very rapid, and antimalarial activity is determined by dihydroartemisinin elimination (half-life approximately 45 min) (40). The extent of protein binding is unknown. No dose modifications are necessary in renal or hepatic impairment.

#### Toxicity

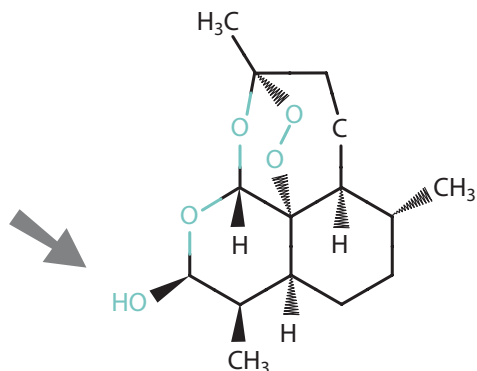
As for artemisinin.

#### Drug interactions

None known.

### A3.6.4 Dihydroartemisinin

Molecular weight: 284.4



Dihydroartemisinin is the main active metabolite of the artemisinin derivatives, but can also be given orally and rectally as a drug in its own right. It is relatively insoluble in water, and requires formulation with suitable excipients to ensure adequate absorption. It achieves cure rates similar to those of oral artesunate. A fixed-dose formulation with piperazine is currently undergoing evaluation as a promising new artemisinin-based combination therapy (ACT).

#### Formulations

- Tablets containing 20 mg, 60 mg or 80 mg of dihydroartemisinin.
- Suppositories containing 80 mg of dihydroartemisinin.

#### Pharmacokinetics

Dihydroartemisinin is rapidly absorbed following oral administration, reaching peak levels after around 2.5 h. Absorption via the rectal route is somewhat slower, with peak levels occurring around 4 h after administration. Plasma protein binding is around 55%. Elimination half-life is approximately 45 min via intestinal and hepatic glucuronidation (48).

#### Toxicity

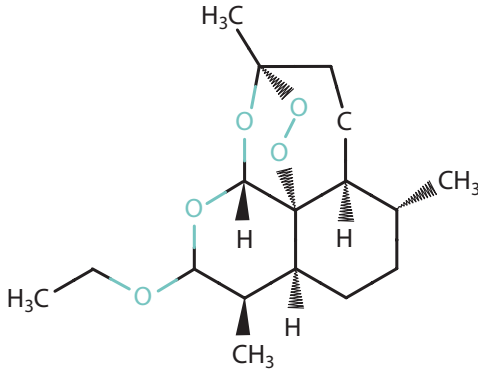
As for artemisinin.

#### Drug interactions

None known.

### A3.6.5 Artemotil

Molecular weight: 312.4



Artemotil, previously known as arteether, is the ethyl ether of artemisinin, and is closely related to the more widely used artemether. It is oil-based so water insoluble. It is given by intramuscular injection only.

#### *Formulations*

- Ampoules containing 150 mg of artemotil in 2 ml of injectable solution.

#### *Pharmacokinetics*

There is less published information on artemotil than for artemether. Absorption is slower and more erratic, with some patients having undetectable plasma artemotil until more than 24 h after administration.

#### *Toxicity*

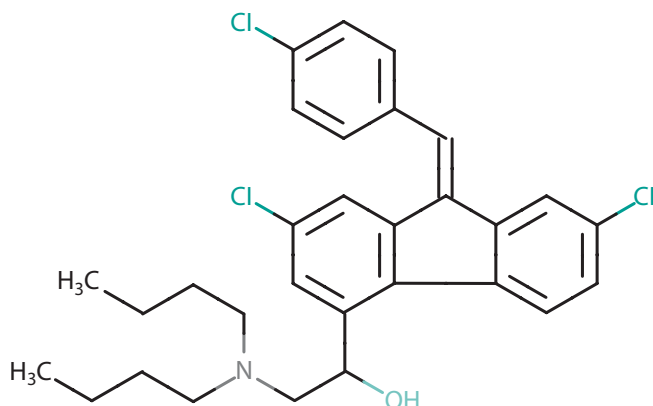
As for artemisinin.

#### *Drug interactions*

None known.

## A3.7 Lumefantrine (benflumetol)

Molecular weight: 528.9



Lumefantrine belongs to the aryl aminoalcohol group of antimalarials, which also includes quinine, mefloquine and halofantrine. It has a similar mechanism of action. Lumefantrine is a racemic fluorine derivative developed in China. It is only available in an oral preparation coformulated with artemether. This ACT is highly effective against multidrug-resistant *P. falciparum*.

### Formulations

Available only in an oral preparation coformulated with artemether:

- Tablets containing 20 mg of artemether and 120 mg of lumefantrine.

### Pharmacokinetics

Oral bioavailability is variable and is highly dependant on administration with fatty foods (38, 49). Absorption increases by 108% after a meal and is lower in patients with acute malaria than in convalescing patients. Peak plasma levels occur approximately 10 h after administration. The terminal elimination half-life is around 3 days.

### Toxicity

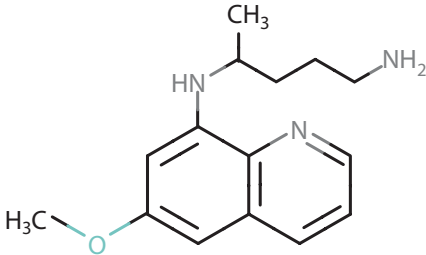
Despite similarities with the structure and pharmacokinetic properties of halofantrine, lumefantrine does not significantly prolong the electrocardiographic QT interval, and has no other significant toxicity (50). In fact the drug seems to be remarkably well tolerated. Reported side effects are generally mild – nausea, abdominal discomfort, headache and dizziness – and cannot be distinguished from symptoms of acute malaria.

### Drug interactions

The manufacturer of artemether-lumefantrine recommends avoiding the following: grapefruit juice; antiarrhythmics, such as amiodarone, disopyramide, flecainide, procainamide and quinidine; antibacterials, such as macrolides and quinolones; all antidepressants; antifungals such as imidazoles and triazoles; terfenadine; other antimalarials; all antipsychotic drugs; and beta blockers, such as metoprolol and sotalol. However, there is no evidence that co-administration with these drugs would be harmful.

## A3.8 Primaquine

Molecular weight: 259.4



Primaquine is an 8-aminoquinoline and is effective against intrahepatic forms of all types of malaria parasite. It is used to provide radical cure of *P. vivax* and *P. ovale* malaria, in combination with a blood schizonticide for the erythrocytic parasites. Primaquine is also gametocytocidal against *P. falciparum* and has significant blood stage activity against *P. vivax* (and some against asexual stages of *P. falciparum*). The mechanism of action is unknown.

### Formulations

- Tablets containing 5.0 mg, 7.5 mg or 15.0 mg of primaquine base as diphosphate.

### Pharmacokinetics

Primaquine is readily absorbed from the gastrointestinal tract. Peak plasma concentrations occur around 1–2 h after administration and then decline, with a reported elimination half-life of 3–6 h (51). Primaquine is widely distributed into body tissues. It is rapidly metabolized in the liver. The major metabolite is carboxyprimaquine, which may accumulate in the plasma with repeated administration.

### Toxicity

The most important adverse effects are haemolytic anaemia in patients with G6PD deficiency, other defects of the erythrocytic pentose phosphate pathway of glucose metabolism, or some other types of haemoglobinopathy (52). In patients with the African variant of G6PD deficiency, the standard course of primaquine generally produces a benign self-limiting anaemia. In the Mediterranean and Asian variants, haemolysis may be much more severe. Therapeutic doses may also cause abdominal pain if administered on an empty stomach. Larger doses can cause nausea and vomiting. Methaemoglobinaemia may occur. Other uncommon effects include mild anaemia and leukocytosis.

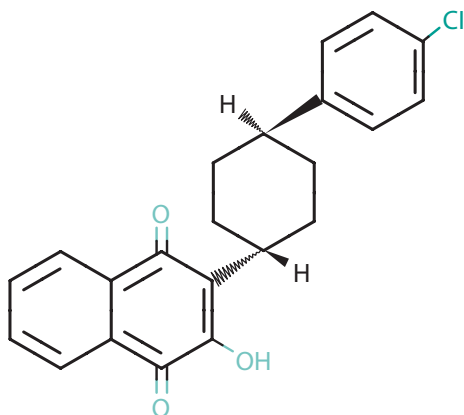
Overdosage may result in leukopenia, agranulocytosis, gastrointestinal symptoms, haemolytic anaemia and methaemoglobinaemia with cyanosis.

### Drug interactions

Drugs liable to increase the risk of haemolysis or bone marrow suppression should be avoided.

## A3.9 Atovaquone

Molecular weight: 366.8



Atovaquone is a hydroxynaphthoquinone antiparasitic drug active against all *Plasmodium* species. It also inhibits pre-erythrocytic development in the liver, and oocyst development in the mosquito. It is combined with proguanil for the treatment of malaria – with which it is synergistic. Atovaquone interferes with cytochrome electron transport.

### Formulations

Atovaquone is available for the treatment of malaria in a co-formulation with proguanil:

- Film-coated tablets containing 250 mg of atovaquone and 100 mg of proguanil hydrochloride for adults.
- Tablets containing 62.5 mg of atovaquone and 25 mg of proguanil hydrochloride for paediatric use.

### Pharmacokinetics

Atovaquone is poorly absorbed from the gastrointestinal tract but bioavailability following oral administration can be improved by taking the drug with fatty foods. Bioavailability is reduced in patients with AIDS. Atovaquone is 99% bound to plasma proteins and has a plasma half-life of around 66–70 h due to enterohepatic recycling. It is excreted almost exclusively in the faeces as unchanged drug. Plasma concentrations are significantly reduced in late pregnancy (53).

### Toxicity

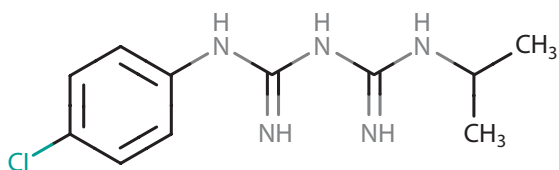
Atovaquone is generally very well tolerated (54). Skin rashes, headache, fever, insomnia, nausea, diarrhoea, vomiting, raised liver enzymes, hyponatraemia and, very rarely, haematological disturbances, such as anaemia and neutropenia, have all been reported.

### Drug interactions

Reduced plasma concentrations may occur with concomitant administration of metoclopramide, tetracycline and possibly also acyclovir, antidiarrhoeal drugs, benzodiazepines, cephalosporins, laxatives, opioids and paracetamol. Atovaquone decreases the metabolism of zidovudine and cotrimoxazole. Theoretically, it may displace other highly protein-bound drugs from plasma-protein binding sites.

## A3.10 Proguanil

Molecular weight: 253.7



Proguanil is a biguanide compound that is metabolized in the body via the polymorphic cytochrome P450 enzyme CYP2C19 to the active metabolite, cycloguanil. Approximately 3% of Caucasian and African populations and 20% of Oriental people are “poor metabolizers” and have considerably reduced biotransformation of proguanil to cycloguanil (55, 56). Cycloguanil inhibits plasmodial dihydrofolate reductase. The parent compound has weak intrinsic antimalarial activity through an unknown mechanism. It is possibly active against pre-erythrocytic forms of the parasite and is a slow blood schizonticide. Proguanil also has sporontocidal activity, rendering the gametocytes non-infective to the mosquito vector. Proguanil is given as the hydrochloride salt in combination with atovaquone. It is not used alone for treatment as resistance to proguanil develops very quickly. Cycloguanil was formerly administered as an oily suspension of the embonate by intramuscular injection.

### Formulations

- Tablets of 100 mg of proguanil hydrochloride containing 87 mg of proguanil base.

In co-formulation with atovaquone:

- Film-coated tablets containing 250 mg of atovaquone and 100 mg of proguanil hydrochloride for adults.
- Tablet containing 62.5 mg of atovaquone and 25 mg of proguanil hydrochloride for paediatric use.

### Pharmacokinetics

Proguanil is readily absorbed from the gastrointestinal tract following oral administration. Peak plasma levels occur at about 4 h, and are reduced in the third trimester of pregnancy. Around 75% is bound to plasma proteins. Proguanil is metabolized in the liver to the active antifolate metabolite, cycloguanil, and peak plasma levels of cycloguanil occur 1 h after those of the parent drug. The elimination half-lives of both proguanil and cycloguanil is

approximately 20 h (57, 58). Elimination is about 50% in the urine, of which 60% is unchanged drug and 30% cycloguanil, and a further amount is excreted in the faeces. Small amounts are present in breast milk. The elimination of cycloguanil is determined by that of the parent compound. The biotransformation of proguanil to cycloguanil via CYP2C19 is reduced in pregnancy and women taking the oral contraceptive pill (59, 60).

### Toxicity

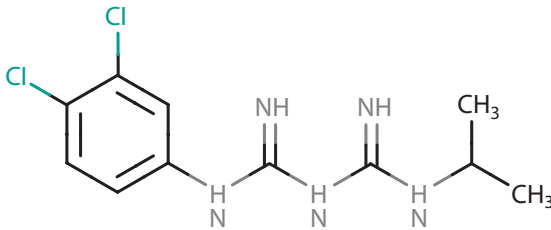
Apart from mild gastric intolerance, diarrhoea, occasional aphthous ulceration and hair loss, there are few adverse effects associated with usual doses of proguanil hydrochloride. Haematological changes (megaloblastic anaemia and pancytopenia) have been reported in patients with severe renal impairment. Overdosage may produce epigastric discomfort, vomiting and haematuria. Proguanil should be used cautiously in patients with renal impairment and the dose reduced according to the degree of impairment.

### Drug interactions

Interactions may occur with concomitant administration of warfarin. Absorption of proguanil is reduced with concomitant administration of magnesium trisilicate.

## A3.11 Chlorproguanil

Molecular weight: 288.2



Chlorproguanil is a biguanide and is given as the hydrochloride salt. Its actions and properties are very similar to those of proguanil. It is available only in combination with a sulfone such as dapsone (co-formulated as Lapdap).

### Pharmacokinetics

Similar to those of proguanil (61).

### Toxicity

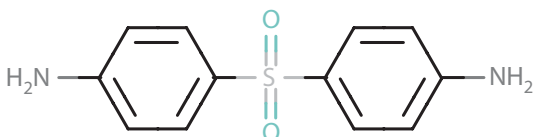
As for proguanil.

### Drug interactions

As for proguanil.

## A3.12 Dapsone

Molecular weight: 248.3



Dapsone is a sulfone widely used for the treatment of leprosy, and sometimes also for treatment or prophylaxis of *Pneumocystis carinii* pneumonia, and treatment of toxoplasmosis, cutaneous leishmaniasis, actinomycetoma and dermatitis herpetiformis. For malaria, dapsone is given in combination with another antimalarial. It is coformulated with chlorproguanil (as Lapdap™). Dapsone inhibits plasmodial dihydropteroate synthase.

### Pharmacokinetics

Dapsone is almost completely absorbed from the gastrointestinal tract, with peak plasma concentrations occurring 2–8 h after an oral dose. Dapsone is 50–80% bound to plasma proteins, as is almost 100% of monoacetyldapsone, its major metabolite. Dapsone undergoes enterohepatic recycling. It is widely distributed to body tissues, including breast milk and saliva. Its elimination half-life is 10–50 h. Dapsone is metabolized by acetylation, which exhibits genetic polymorphism. Hydroxylation is the other metabolic pathway, resulting in hydroxylamine dapsone, which may be responsible for dapsone-associated methaemoglobinaemia and haemolysis. Dapsone is mainly excreted in the urine, only 20% as unchanged drug.

### Toxicity

Varying degrees of haemolysis and methaemoglobinaemia are the most frequently reported adverse effects and occur in most patients given more than 200 mg of dapsone daily. Doses of up to 100 mg daily do not cause significant haemolysis but patients deficient in G6PD are affected by doses of >50 mg daily. Haemolytic anaemia has been reported following ingestion of dapsone in

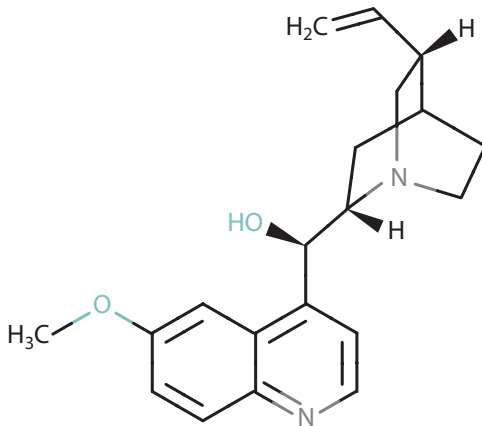
breast milk. Agranulocytosis has been reported following use of dapsone and pyrimethamine together as malaria prophylaxis – particularly when used twice weekly. Aplastic anaemia has also been reported. Rashes, including pruritus and fixed-drug reactions may occur but serious cutaneous hypersensitivity is rare. “Dapsone syndrome” consists of rash, fever, jaundice and eosinophilia, and has been reported in a few patients using dapsone as malaria prophylaxis, but mainly in leprosy patients on long treatment courses. Other rare adverse effects include anorexia, nausea, vomiting, headache, hepatitis, hypoalbuminaemia and psychosis.

### Drug interactions

There is an increased risk of dapsone toxicity with concomitant administration of probenecid, trimethoprim and amprenovir. Levels of dapsone are reduced with rifampicin.

## A3.13 Quinine

Molecular weight: 324.4



Quinine is an alkaloid derived from the bark of the *Cinchona* tree. Four anti-malarial alkaloids can be derived from the bark: quinine (the main alkaloid), quinidine, cinchonine and cinchonidine. Quinine is the L-stereoisomer of quinidine.

Quinine acts principally on the mature trophozoite stage of parasite development and does not prevent sequestration or further development of circulating ring stages of *P. falciparum*. Like other structurally similar antimalarials, quinine also kills the sexual stages of *P. vivax*, *P. malariae* and *P. ovale*, but not mature

gametocytes of *P. falciparum*. It does not kill the pre-erythrocytic stages of malaria parasites. The mechanisms of its antimalarial actions are thought to involve inhibition of parasite haem detoxification in the food vacuole, but are not well understood.

### Formulations

- Tablets of quinine hydrochloride, quinine dihydrochloride, quinine sulfate and quinine bisulfate containing 82%, 82%, 82.6% and 59.2% quinine base, respectively.
- Injectable solutions of quinine hydrochloride, quinine dihydrochloride and quinine sulfate containing 82%, 82% and 82.6% quinine base, respectively.

### Pharmacokinetics

The pharmacokinetic properties of quinine are altered significantly by malaria infection, with reductions in apparent volume of distribution and clearance in proportion to disease severity (16, 62). In children under 2 years of age with severe malaria, concentrations are slightly higher than in older children and adults (63). There is no evidence for dose-dependent kinetics. Quinine is rapidly and almost completely absorbed from the gastrointestinal tract and peak plasma concentrations occur 1–3 h after oral administration of the sulfate or bisulfate (64). It is well absorbed after intramuscular injection in severe malaria (65, 66). Plasma-protein binding, mainly to alpha 1-acid glycoprotein, is 80% in healthy subjects but rises to around 90% in patients with malaria (67–69). Quinine is widely distributed throughout the body including the cerebrospinal fluid (2–7% of plasma values), breast milk (approximate 30% of maternal plasma concentrations) and the placenta (70). Extensive metabolism via the cytochrome P<sub>450</sub> enzyme CYP<sub>3A4</sub> occurs in the liver and elimination of more polar metabolites is mainly renal (71, 72). The initial metabolite 3-hydroxyquinine contributes approximately 10% of the antimalarial activity of the parent compound, but may accumulate in renal failure (73). Excretion is increased in acid urine. The mean elimination half-life is around 11 h in healthy subjects, 16 h in uncomplicated malaria and 18 h in severe malaria (62). Small amounts appear in the bile and saliva.

### Toxicity

Administration of quinine or its salts regularly causes a complex of symptoms known as cinchonism, which is characterized in its mild form by tinnitus, impaired high tone hearing, headache, nausea, dizziness and dysphoria, and sometimes disturbed vision (7). More severe manifestations include vomiting, abdominal pain, diarrhoea and severe vertigo. Hypersensitivity reactions to

quinine range from urticaria, bronchospasm, flushing of the skin and fever, through antibody-mediated thrombocytopenia and haemolytic anaemia, to life-threatening haemolytic-uraemic syndrome. Massive haemolysis with renal failure (“black water fever”) has been linked epidemiologically and historically to quinine, but its etiology remains uncertain (74). The most important adverse effect in the treatment of severe malaria is hyperinsulinaemic hypoglycaemia (75). This is particularly common in pregnancy (50% of quinine-treated women with severe malaria in late pregnancy). Intramuscular injections of quinine dihydrochloride are acidic (pH 2) and cause pain, focal necrosis and in some cases abscess formation, and in endemic areas are a common cause of sciatic nerve palsy. Hypotension and cardiac arrest may result from rapid intravenous injection. Intravenous quinine should be given only by infusion, never injection. Quinine causes an approximately 10% prolongation of the electrocardiograph QT interval – mainly as a result of slight QRS widening (75). The effect on ventricular repolarization is much less than that with quinidine. Quinine has been used as an abortifacient, but there is no evidence that it causes abortion, premature labour or fetal abnormalities in therapeutic use.

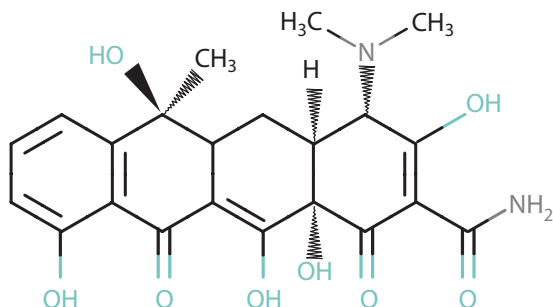
Overdosage of quinine may cause oculotoxicity, including blindness from direct retinal toxicity, and cardiotoxicity, and can be fatal (76). Cardiotoxic effects are less frequent than those of quinidine and include conduction disturbances, arrhythmias, angina, hypotension leading to cardiac arrest and circulatory failure. Treatment is largely supportive, with attention being given to maintenance of blood pressure, glucose and renal function, and to treating arrhythmias.

### *Drug interactions*

There is a theoretical concern that drugs that may prolong the QT interval should not be given with quinine, although whether or not quinine increases the risk of iatrogenic ventricular tachyarrhythmia has not been established. Antiarrhythmics, such as flecainide and amiodarone, should probably be avoided. There might be an increased risk of ventricular arrhythmias with antihistamines such as terfenadine, and with antipsychotic drugs such as pimozide and thioridazine. Halofantrine, which causes marked QT prolongation, should be avoided but combination with other antimalarials, such as lumefantrine and mefloquine is safe. Quinine increases the plasma concentration of digoxin. Cimetidine inhibits quinine metabolism, causing increased quinine levels and rifampicin increases metabolic clearance leading to low plasma concentrations and an increased therapeutic failure rate (77).

## A3.14 Tetracycline

Molecular weight: 444.4



The tetracyclines are a group of antibiotics originally derived from certain *Streptomyces* species, but used mostly in synthetic form. Tetracycline itself may be administered orally or intravenously as the hydrochloride salt or phosphate complex. Both are water soluble, although the intravenous preparation is only stable for a few hours. Tetracyclines are inhibitors of aminoacyl-tRNA binding during protein synthesis. They have a broad range of uses, including treatment of some bacterial infections: *Chlamydia*, *Rickettsia*, *Mycoplasma*, Lyme disease, *Brucella*, tularaemia, plague and cholera. Doxycycline is a synthetic tetracycline with a longer half-life, which makes dosing schedules easier.

### Formulations

- Capsules and tablets containing 250 mg of tetracycline hydrochloride, equivalent to 231 mg of tetracycline base.

### Pharmacokinetics

Some 60–80% of tetracycline is absorbed from the gastrointestinal tract following oral administration. Absorption is reduced by the presence of divalent and trivalent metal ions with which it forms stable, insoluble complexes. Thus absorption may be impaired with food or milk. Formulation with phosphate may improve absorption. Peak plasma concentrations occur 1–3 h after ingestion. Tetracycline is 20–65% bound to plasma proteins. It is widely distributed throughout the body, although less so than the more lipophilic doxycycline. High concentrations are present in breast milk (around 60% of plasma levels), and also diffuse readily across the placenta, and are retained in sites of new bone formation and teeth development. The half-life of tetracycline is around 8 h;

40–70% is excreted in the urine via glomerular filtration. The remainder is excreted in the faeces and bile. Enterohepatic recycling slows down elimination.

### Toxicity

All the tetracyclines have similar adverse effect profiles. Gastrointestinal effects, such as nausea, vomiting and diarrhoea, are common, especially with higher doses, and are due to mucosal irritation. Dry mouth, glossitis, stomatitis, dysphagia and oesophageal ulceration have also been reported. Overgrowth of *Candida* and other bacteria occurs, presumably due to disturbances in gastrointestinal flora as a result of incomplete absorption of the drug. This effect is seen less frequently with doxycycline, which is better absorbed. Pseudomembranous colitis, hepatotoxicity and pancreatitis have also been reported.

Tetracyclines accumulate in patients with renal impairment and this may cause renal failure. In contrast doxycycline accumulates less and is preferred in patient with renal impairment. The use of out-of-date tetracycline can result in the development of a reversible Fanconi-type syndrome characterized by polyuria and polydipsia with nausea, glycosuria, aminoaciduria, hypophosphataemia, hypokalaemia, and hyperuricaemia with acidosis and proteinuria. These effects have been attributed to the presence of degradation products, in particular anhydroepitetracycline.

Tetracyclines are deposited in deciduous and permanent teeth during their formation and cause discoloration and enamel hypoplasia. They are also deposited in calcifying areas in bone and the nails and interfere with bone growth in young infants or pregnant women. Raised intracranial pressure in adults and infants has also been documented. Tetracyclines use in pregnancy has also been associated with acute fatty liver. Tetracyclines should therefore not be given to pregnant or lactating women, or children of less than 8 years of age.

Hypersensitivity reactions occur, although they are less common than for  $\beta$ -lactam antibiotics. Rashes, fixed drug reactions, drug fever, angioedema, urticaria, pericarditis and asthma have all been reported. Photosensitivity may develop and, rarely, haemolytic anaemia, eosinophilia, neutropenia and thrombocytopenia. Pre-existing systemic lupus erythematosus may be worsened and tetracyclines are contraindicated in patients with the established disease.

### Drug interactions

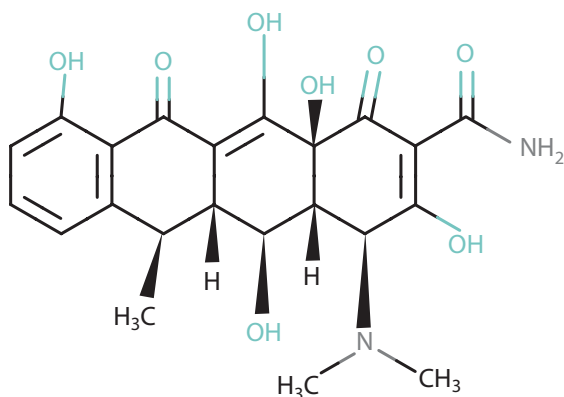
There is reduced absorption of tetracyclines with concomitant administration of cations, such as aluminium, bismuth, calcium, iron, zinc and magnesium. Administration with antacids, iron preparations, dairy products and some

other foods should therefore be avoided. Nephrotoxicity may be exacerbated with diuretics, methoxyflurane or other potentially nephrotoxic drugs. Potentially hepatotoxic drugs should be avoided. Tetracyclines produce increased concentrations of digoxin, lithium and theophylline, and decrease plasma atovaquone concentrations and also the effectiveness of oral contraceptives. They may antagonize the actions of penicillins so should not be given concomitantly.

### A3.15 Doxycycline

(See also tetracycline)

Molecular weight: 444.4



Doxycycline is a tetracycline derivative with uses similar to those of tetracycline. It may be preferred to tetracycline because of its longer half-life, more reliable absorption and better safety profile in patients with renal insufficiency, where it may be used with caution. It is relatively water insoluble but very lipid soluble. It may be given orally or intravenously. It is available as the hydrochloride salt or phosphate complex, or as a complex prepared from the hydrochloride and calcium chloride.

#### Formulations

- Capsules and tablets containing 100 mg of doxycycline salt as hydrochloride.

#### Pharmacokinetics

Doxycycline is readily and almost completely absorbed from the gastrointestinal tract and absorption is not affected significantly by the presence of food. Peak plasma concentrations occur 2 h after administration. Some 80–95% is protein-

bound and half-life is 10–24 h (78). It is widely distributed in body tissues and fluids. In patients with normal renal function, 40% of doxycycline is excreted in the urine, although more if the urine is alkalinized. It may accumulate in renal failure. However, the majority of the dose is excreted in the faeces.

### Toxicity

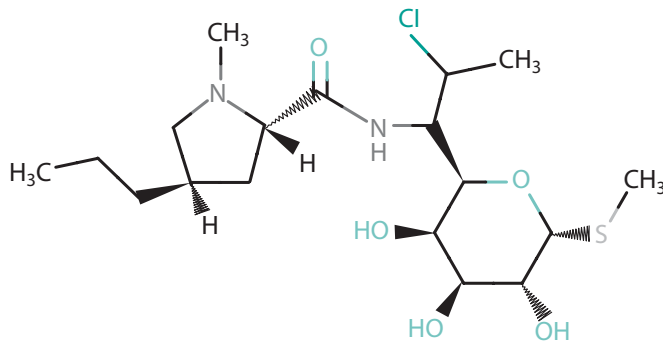
As for tetracycline. Gastrointestinal effects are fewer than with tetracycline, although oesophageal ulceration can still be a problem if insufficient water is taken with tablets or capsules. There is less accumulation in patients with renal impairment. Doxycycline should not be given to pregnant or lactating women, or children aged up to 8 years.

### Drug interactions

Doxycycline has a lower affinity for binding with calcium than other tetracyclines, so may be taken with food or milk. However, antacids and iron may still affect absorption. Metabolism may be accelerated by drugs that induce hepatic enzymes, such as carbamazepine, phenytoin, phenobarbital and rifampicin, and by chronic alcohol use.

## A3.16 Clindamycin

Molecular weight: 425.0



Clindamycin is a lincosamide antibiotic, i.e. a chlorinated derivative of lincomycin. It is very soluble in water. It inhibits the early stages of protein synthesis by a mechanism similar to that of the macrolides. It may be administered by mouth as capsules containing the hydrochloride or as oral liquid preparations containing the palmitate hydrochloride. Clindamycin is given parenterally as the phosphate either by the intramuscular or the intravenous

route. It is used for the treatment of anaerobic and Gram-positive bacterial infections, babesiosis, toxoplasmosis and *Pneumocystis carinii* pneumonia.

### Formulations

- Capsules containing 75 mg, 150 mg or 300 mg of clindamycin base as hydrochloride.

### Pharmacokinetics

About 90% of a dose is absorbed following oral administration. Food does not impede absorption but may delay it. Clindamycin phosphate and palmitate hydrochloride are rapidly hydrolysed to form the free drug. Peak concentrations may be reached within 1 h in children and 3 h in adults. It is widely distributed, although not into the cerebrospinal fluid. It crosses the placenta and appears in breast milk. It is 90% bound to plasma proteins and accumulates in leukocytes, macrophages and bile. The half-life is 2–3 h but this may be prolonged in neonates and patients with renal impairment. Clindamycin undergoes metabolism to the active *N*-demethyl and sulfoxide metabolites, and also some inactive metabolites. About 10% of a dose is excreted in the urine as active drug or metabolites and about 4% in the faeces. The remainder is excreted as inactive metabolites. Excretion is slow and takes place over many days. Clindamycin is not effectively removed from the body by dialysis.

### Toxicity

Diarrhoea occurs in 2–20% of patients. In some, pseudomembranous colitis may develop during or after treatment, which can be fatal. Other reported gastrointestinal effects include nausea, vomiting, abdominal pain and an unpleasant taste in the mouth. Around 10% of patients develop a hypersensitivity reaction. This may take the form of skin rash, urticaria or anaphylaxis. Other adverse effects include leukopenia, agranulocytosis, eosinophilia, thrombocytopenia, erythema multiforme, polyarthrititis, jaundice and hepatic damage. Some parenteral formulations contain benzyl alcohol, which may cause fatal “gasping syndrome” in neonates.

### Drug interactions

Clindamycin may enhance the effects of drugs with neuromuscular blocking activity and there is a potential danger of respiratory depression. Additive respiratory depressant effects may also occur with opioids. Clindamycin may antagonize the activity of parasympathomimetics.

## A3.17 Pharmacology of antimalarials in special groups and conditions

### A3.17.1 Safety and tolerability of antimalarials in infants

Infants under 12 months of age constitute a significant proportion of patients in malaria endemic countries. Yet few studies focus specifically on this age range, partly because of ethical dilemmas and also owing to technical difficulties with sampling. Very young children cannot report adverse effects themselves, so detection of these is dependent upon parents and health professionals making observations. In addition, pre-marketing clinical trials for new drugs are not represented by important subpopulations including infants (79), yet there are potentially important pharmacokinetic differences in infants compared to older children and adults (80).

#### *Drug absorption*

The gastric pH at birth is usually 6–8 but within a few hours falls to 2 and then rises again until virtual achlorhydria occurs for several days. As the gastric mucosa develops, the acidity increases again until 3 years of age when adult values are attained. The gastric emptying time is prolonged (up to 8 h) in neonates and approaches adult values only after 6 months. Intramuscular injections can also be problematic in young children. Infants with acute or severe malaria may become extremely “shut down” whereby visceral, muscle and skin blood flow is reduced. This may result in slow, erratic or incomplete drug absorption and the consequent delay in achieving therapeutic drug levels at a time when speed and adequacy of drug delivery are crucial.

#### *Distribution*

Relatively large total and extracellular body water compartments in infancy lead to larger apparent volumes of distribution. Total body lipids rise steadily after birth for the first 9 months of life but then decrease until adolescence. These changes in body composition can modulate volume of distribution and clearance. Liver mass per body weight is higher in infants than adults and the liver undergoes rapid growth during the first 2 years. The brain is disproportionately large in young children, and the blood brain barrier relatively immature, making a further contribution to volume of distribution. Finally drug distribution is also affected by lower protein binding in infancy with more free drug and thus increased clearance. The former might also lead to a greater risk of toxicity.

### *Drug metabolism*

The cytochrome P<sub>450</sub> mixed function oxidase system is the most important biotransformation system incorporating many enzymes and isoenzymes. In general, these enzyme systems are immature at birth. There is therefore relatively slow clearance of most metabolized drugs in the first 2–3 months of life. Between 2 and 6 months clearance is more rapid than in adults and even more so for most drugs from 6 months to 2 years (elimination half-life for metabolized drugs in infants aged 6 months to 2 years is 0.6 times that in adults).

### *Renal clearance*

Glomerular filtration rate only reaches surface-area-adjusted adult levels at around 6 months of age. Thus for drugs that rely on renal elimination, elimination half-lives in very young infants may be up to 2–3 times longer than in adults. After 2 months, half-lives are shorter (0.35–0.5 times adult values) until about 2 years of age.

## **A3.17.2 Malnutrition and antimalarials**

Malaria and malnutrition frequently coexist. The relationship between malaria and nutritional status is complex and has been the subject of debate for many years (81). Given that a significant proportion of the world's malnourished children live in malaria endemic countries (82) it is important to understand how antimalarial drug disposition may be affected when malnutrition is severe. This section outlines the physiological changes that occur in malnourished patients and discusses how these may influence the pharmacokinetic properties of antimalarials, drawing on the few studies of antimalarial drug disposition in malnutrition that are available

in reviewing the literature it was apparent that many studies were conducted in populations and settings where some degree of malnutrition would have been expected. However, this was only rarely mentioned as a possible confounder for drug efficacy, although there was an occasional comment that obviously malnourished patients appeared to respond differently to treatment than did other patients (83). Several ongoing studies are planning to look specifically at treatment outcomes in this group of patients.

### *Definitions*

There are different ways of classifying malnutrition. Earlier studies employ the Wellcome classification: where body weight is given as a percentage of standard weight (50th percentile of the Harvard value): underweight 80–60%; marasmus 60%; kwashiorkor 80–60% + oedema; marasmic kwashiorkor 60% + oedema. Other studies refer to low weight-for-height (wasting); low weight-

for-age (underweight); or low height-for-age (stunting) and use anthropometric indicators and reference standards. Protein-energy malnutrition is defined as a range of pathological conditions arising from coincident lack, in varying proportions, of protein and calories, occurring most frequently in infants and young children and commonly associated with infections (84).

### Pharmacokinetics

Anorexia, diarrhoea and vomiting are common. Anorexia will affect the absorption of drugs requiring concomitant administration of fatty foods, and oral bioavailability will be reduced in vomiting patients or those with a rapid transit time. Atrophy of the bowel mucosa, which occurs in severe protein-energy malnutrition, will also hinder absorption.

Children with oedematous lower limbs may be expected to have altered absorption from intramuscular injections. Patients with protein-energy malnutrition frequently have poor peripheral perfusion due to circulatory insufficiency associated with bradycardia, hypotension and a reduced cardiac output. Thus absorption of intramuscular and possibly intrarectal drugs may be expected to be slower than in patients without protein-energy malnutrition. Diminished muscle mass may make repeated intramuscular injections difficult.

Total body water increases in proportion to the degree of malnutrition, mainly owing to an expansion of the extracellular fluid (most obvious in oedematous patients). Thus the volume of distribution of some drugs can be expected to be larger and plasma concentrations lower. Albumin is the most important plasma protein for binding of many drugs, but in protein-energy malnutrition hypoalbuminaemia results from decreased synthesis as dietary deficiency occurs. With highly bound drugs this could in theory lead to an increase in the amount of unbound drug, which may increase both the elimination, since more drug is available for metabolism, and potential toxicity. There are other plasma proteins less severely affected by decreased synthesis, and if these are able to bind some free drug, then the increase in free fraction might not be as great as anticipated.

Fatty infiltration occurs but jaundice is uncommon unless septicaemia is present. Liver function tests may be abnormal and urea cycle enzymes are decreased. Children with kwashiorkor excreted a higher proportion of unchanged chloroquine

before therapy than in the recovery phase (85). This suggests that hepatic function was inadequate during the acute phase of kwashiorkor. Animal studies have demonstrated that some enzyme systems, such as cytochrome P450, have decreased activity in the presence of significant malnutrition.

Owing to the reduction in cardiac output, the kidneys receive less than the usual 25% of renal blood flow. Glomerular filtration rate, renal blood flow and tubular function have all been shown to be inadequate, and compounded by concomitant dehydration. Drugs dependent on renal excretion might be expected to have elevated plasma concentrations under such circumstances. Abnormal excretion of drugs into bile has also been described in severe protein-energy malnutrition.

### *Antimalarials and protein-energy malnutrition*

Few data are available for chloroquine kinetics in malnourished patients. Children with kwashiorkor excreted a higher ratio of chloroquine to its metabolites before nutritional rehabilitation (85). Presumably the metabolism of chloroquine by the liver was affected adversely in protein-energy malnutrition. In a study of chloroquine pharmacokinetics in five children with kwashiorkor (but without malaria), peak plasma concentrations of the drug were approximately one-third of the values for healthy controls (mean  $40 \pm 30$  ng/ml compared to  $134 \pm 99$  ng/ml), but the times to peak levels and the elimination half-lives were not significantly different, indicating reduced absorption. There was also reduced metabolism of chloroquine to its metabolite, desethylchloroquine, which suggested some impairment of drug metabolism. However, the study did not consider plasma protein binding or drug distribution. Currently there are no recommendations for dose alterations in patients with protein-energy malnutrition (86).

Three studies examining the kinetics of quinine in malnourished patients have been published. The first from Nigeria compared the pharmacokinetics of an oral dose of quinine 10 mg/kg in six children with kwashiorkor and seven normal controls who were attending a malaria follow-up clinic (87). The children were aged 1–3 years. Values for total plasma proteins and albumin for children with kwashiorkor were 74% and 67% of those for control children. Absorption of quinine was slower in the kwashiorkor group than in the controls (mean time to maximum concentration ( $t_{max}$ )  $2.5 \pm 0.3$  h compared to  $1.5 \pm 0.6$  h);

maximum plasma concentration ( $C_{\max}$ ) was also lower ( $1.7 \pm 0.5 \mu\text{mol/l}$  compared to  $2.4 \pm 0.3 \mu\text{mol/l}$ ). Rate of clearance of quinine in kwashiorkor was less than one-third of the value for well-nourished patients ( $31.5 \pm 8.5 \text{ mg/min}$  compared to  $108.5 \pm 34.8 \text{ mg/min}$ ) and the elimination half-life was also longer ( $15.0 \pm 4.4 \text{ h}$  compared to  $8.0 \pm 1.3 \text{ h}$ ). The authors concluded that the combination of malabsorption, reduced plasma protein binding and reduced metabolism in the liver was responsible for the differences observed. No dose alterations were suggested.

The second study, in Gabon, compared eight children with non-kwashiorkor global malnutrition (defined as having a ratio of left mid-arm circumference:head circumference  $< 0.279$ ) with seven children with normal nutritional status (88). The children were aged 9–60 months. Only two were subsequently confirmed to have malaria, although all had been febrile at presentation. Mean serum albumin levels in the two groups were 28.7 and 31.0 respectively. Each child received a loading dose of 16 mg/kg quinine base (25 mg/kg quinine resorcine hydrochloride; Quinimax) by deep intramuscular injection followed by 8 mg/kg at 12 h. The  $t_{\max}$  was significantly shorter in malnourished children ( $1.1 \pm 0.4 \text{ h}$  compared to  $2.2 \pm 1.2 \text{ h}$ ). No difference was observed for  $C_{\max}$ , volume of distribution or protein binding. Clearance was significantly faster for malnourished children ( $4.4 \pm 3.6 \text{ ml/min/kg}$  compared to  $2.3 \pm 1.4 \text{ ml/min/kg}$ ), and half-life shorter ( $6.3 \pm 1.8 \text{ h}$  compared to  $10.1 \pm 3.4 \text{ h}$ ). Concentration at 12 h was lower in malnourished children ( $3.3 \pm 1.6 \text{ mg/ml}$  compared to  $5.3 \pm 1.6 \text{ mg/l}$ ). There was a significant correlation between elimination half-life and left mid-arm:head circumference. The ratio between the area under the curve for hydroxyquinine, the main metabolite of quinine, and that for quinine was significantly higher in the malnourished group and significantly correlated with left mid-arm:head circumference ratio, indicating increased metabolism of quinine in malnourished patients. The authors suggest that the administration interval should be reduced to 8 h in malnourished children in order to obtain plasma concentrations of quinine similar to those found in children with normal nutrition.

In the third study, from Niger, 40 children were divided into four groups: normally nourished children with or without cerebral malaria, and malnourished children ( $>2$  SD below the median value for at least two of the following: weight-for-height, weight-for-age and height-for-age) with or without cerebral malaria (89). The age range studied was 24–72 months. Patients with kwashiorkor were excluded. All patients received 4.7 mg/kg quinine base (as 8 mg/kg Quinimax) by intravenous infusion over 4 h. Infusions were repeated every 8 h for children with cerebral malaria.  $C_{\max}$  was highest in malnourished children, and was higher in those without malaria than with malaria ( $8.5 \pm 4.7 \text{ mg/l}$  compared to  $7.7 \pm 2.0 \text{ mg/l}$ ); it was lowest in the control groups without and with malaria

( $3.0 \pm 2.1$  mg/l and  $6.6 \pm 3.0$  mg/l). There were no differences between the area under the curve for 0–8 h and elimination half-life for the two malnutrition groups and controls with malaria, but all were higher than for controls without malaria. Conversely plasma clearance of quinine and volume of distribution were smaller in these three groups than in controls without malaria. Alpha 1-glycoprotein plasma concentrations and protein-bound fraction of the drug were increased in the three groups. Malnourished children had slower parasite clearance but the difference was not significant. The authors concluded that severe global malnutrition and cerebral malaria have a similar effect on quinine pharmacokinetics in children and that cerebral malaria-mediated modifications of quinine disposition are not potentiated. They recommend that current dosing schedules should not be altered for children with malnutrition.

No studies exist of sulfadoxine-pyrimethamine kinetics in malnourished patients. However, observational data from Rwandan refugee children showed that malnourished children (defined as weight-for-height < 80% of the reference median with or without oedema) were more likely to have treatment failure than children without malnutrition (86% compared to 58% (83). Higher initial parasite counts and host immunity, as well as pharmacokinetic differences, may also have contributed to this finding.

A number of small studies have been conducted on tetracycline kinetics in malnourished adults from India. One study compared the kinetics of intravenous and oral tetracycline in malnourished and normal adult males (90). Compared to the control group, malnourished patients had lower protein binding, shorter elimination half-life and reduced volume of distribution. The authors suggest that in order to keep levels of tetracycline above the minimum inhibitory concentration, the dose interval should be reduced. A similar conclusion was reached by another study that also found more rapid distribution of tetracycline and faster clearance in the malnourished group (91). The same author, in a separate study, also looked at absorption of oral compared with intravenous tetracycline in various types of malnutrition. Oral absorption was slower in patients with protein-energy-malnutrition and pellagra than in patients with anaemia or vitamin B complex deficiency patients and healthy controls. In a third study, patients with nutritional oedema were found to have increased  $C_{max}$  and area under the curve values, and reduced clearance and volume of distribution compared with healthy controls (i.e. some differences with non-oedema malnutrition patients) (92).

There is a single study examining the kinetics of doxycycline given orally to adult patients in India (93). Area under the curve, elimination half-life and plasma protein binding were reduced, and clearance increased in the malnourished group. Renal clearance was similar in controls and malnourished patients. The authors surmised that increased total body clearance of doxycycline might be due to higher metabolism in malnourished patients. Steady state plasma  $C_{\min}$  levels were lower than in healthy patients but still within the therapeutic range. A change in dose recommendation does not seem necessary given these findings.

There are no studies of the kinetics of clindamycin, amodiaquine, artemisinin derivatives (dihydroartemisinin), artemether-lumefantrine, mefloquine or primaquine kinetics in malnourished patients.

### Conclusion

There are many reasons why pharmacokinetics may be different in malnourished patients compared to those who are well nourished. However, with the possible exception of quinine, there are insufficient data available for specific dosing changes to be recommended.

## A3.18 References<sup>24</sup>

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<sup>24</sup> Further information on the chemistry and pharmacology of antimalarials can be obtained from the web site of the United States National Library of Medicines, Specialized Information Services, ChemIDplus Advanced: <http://chem.sis.nlm.nih.gov/chemidplus>.

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## ANNEX 4

### ANTIMALARIALS AND MALARIA TRANSMISSION

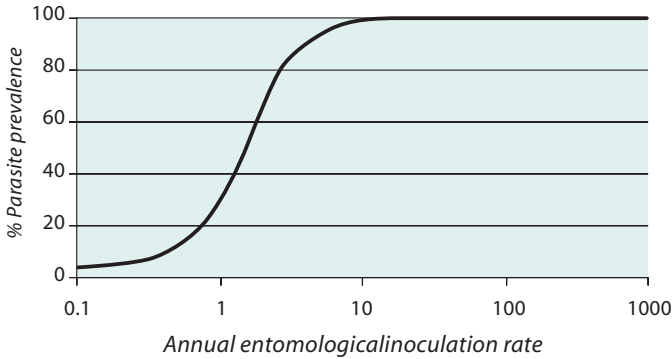
#### A4.1 Principles of malaria transmission

Malaria is spread from person to person by mosquitoes belonging to the genus *Anopheles*. The female mosquito is infected by the sexual stages of the parasite, the gametocytes, when it bites a malaria-infected person to take a blood meal. The gametocytes undergo further development in the insect for a period of 6–12 days, after which they are capable of infecting a human, again through the bite of the mosquito.

The intensity of malaria transmission in an area is the rate at which people are inoculated with malaria parasites by mosquitoes. It is usually expressed as the annual entomological inoculation rate (EIR), i.e. the average number of infectious bites by malaria-infected mosquitoes delivered to an individual human resident in the area in the period of one year. It is the EIR that determines, to a large extent, the epidemiology of malaria and the pattern of clinical disease in that locality. The high end of the malaria transmission range is found in a few parts of tropical Africa, where EIRs of 500–1000 can be reached (1). At the low end of the range are EIRs of 0.01 or below, as found in the temperate climates of the Caucasus and Central Asia where malaria transmission is only barely sustained. Between these extremes are situations of unstable seasonal malaria such as in much of Asia and Latin America where EIRs lie below 10, and often around 1–2, and situations of stable but still seasonal malaria as in much of West Africa where the EIR is in the range 10–100.

The proportion of infected mosquitoes in a locality is itself related to the number of infected and infectious humans living there. Therefore, lowering of the infectivity of infected persons to the mosquito vector will contribute to lowering of malaria transmission, and eventually to reducing the prevalence of malaria and the incidence of disease in that locality. The relationship between the EIR and the prevalence of malaria is, however, complex and is affected by the nature of immunity to malaria, its acquisition and loss and to whether or not there is effective drug treatment. The hypothetical relationship represented in figure A4.1 assumes no drug treatment. In areas of low transmission, where EIRs are below 1 or 2, a reduction in the inoculation rates will result in an almost proportionate reduction in the prevalence (and incidence rate) of malaria. At EIRs in excess of 10, the reductions in transmission need to be increasingly large if they are to make a significant impact on

malaria prevalence. In high-transmission settings where there is great redundancy in the infectious reservoir, the impact of reducing transmission on disease incidence is not at all obvious, and has been the subject of considerable debate. The experience with major interventions, such as the use of insecticide-treated nets, suggests, however, that effective transmission-reducing interventions will always be beneficial with respect to mortality (2, 3).



**Figure A4.1** Relationship between inoculation rate and parasite prevalence (assumes that all infections are untreated)

## A4.2 Effects of antimalarials on malaria transmission

Antimalarials can help bring about a reduction in malaria transmission by their effect on parasite infectivity. This can be a direct effect on the gametocytes, the infective stages found in human infections (gametocytocidal effect) or, when the drug is taken up in the blood meal of the mosquito, an effect on the parasite's development in the insect (sporonticidal effect) (Table A4.1; Figure A4.2). Chloroquine acts against young gametocytes but has no suppressive effects on mature infective forms (4). Chloroquine has even been shown to be capable of enhancing the infectivity of gametocytes to the mosquito (5). In contrast, sulfadoxine-pyrimethamine increases gametocyte carriage but, provided there is no resistance, reduces the infectivity of gametocytes to mosquitoes (5–7). Artemisinins are the most potent gametocytocidal drugs among those currently being used to treat an asexual blood infection (8–11). They destroy immature gametocytes, preventing new infective gametocytes from entering the circulation, but their effects on mature gametocytes are less and so they will not affect the infectivity of those already present in the circulation at the time a patient presents for treatment (11).

Table A4.1

*P. falciparum*

Drug	Effect of treatment			
	Gametocytocidal		Sporonticidal	
	Viability of young sequestered gametocytes	Viability of mature circulating gametocytes	Infectivity of gametocytes to mosquitoes	Overall effect on suppressing infectivity <sup>a</sup>
Chloroquine	Reduces	No effect (4)	Enhances (5)	+
Sulfadoxine-pyrimethamine	No effect	Increases (5–7)	Suppresses (5–7)	+/-
Artemisinin derivatives	Reduces greatly (8–11)	Little effect (11)	Unknown	+++
Primaquine	Unknown	Reduces greatly (11)	Unknown	+++
Quinine (4)	No effect	No effect	No effect	None

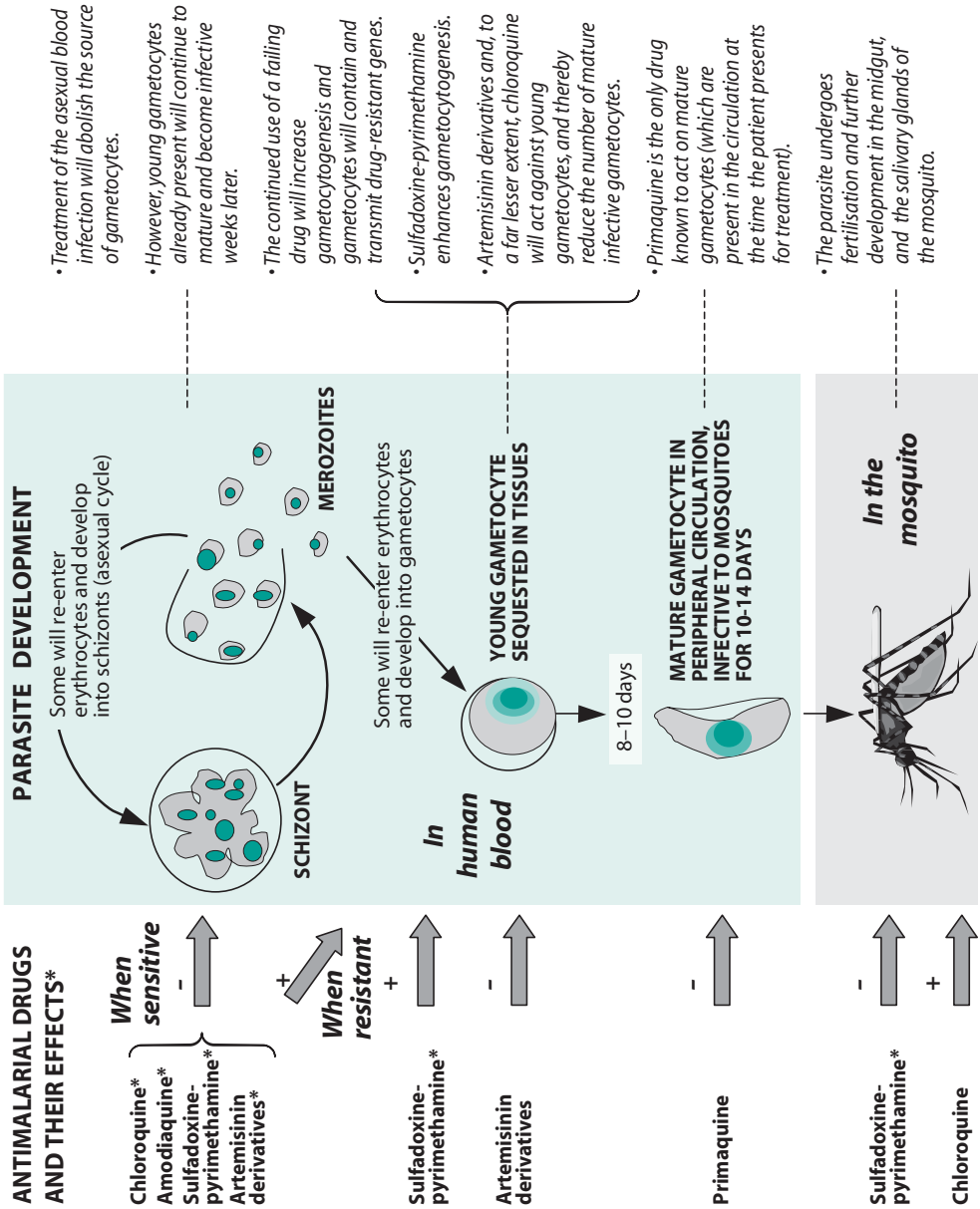
<sup>a</sup> +/- no overall effect; + moderate effect; ++ high effect; +++ very high effect

Primaquine, an 8-aminoquinoline antimalarial that has been widely used as a hypnozoiticidal drug, is the only antimalarial medicine that had been deployed in the treatment of *P. falciparum* infections specifically for its effects on infectivity. It acts on mature infective gametocytes in the circulation and accelerates gametocyte clearance (11), as opposed to artemisinins which mainly inhibit gametocyte development.

## A4.3 The use of antimalarials to reduce infectivity

### A4.3.1 Choice of drugs

Artemisinin derivatives, as indicated earlier, have specific and significant activity against gametocytes (Table A3.1). Effective treatment of a malaria blood infection with any antimalarial will, nevertheless, remove the source of new gametocytes by eliminating the asexual blood stages from which gametocytes derive. The faster the clearance of asexual blood parasites by a drug, the greater will be its impact on infectivity. In *P. vivax*, *P. malariae* and *P. ovale* infections, in which gametocytes have a short developmental period and are short-lived, effective treatment of the asexual blood infection alone (without the addition of gametocytocidal drugs) will be sufficient to abolish further infectivity to mosquitoes. *P. falciparum* is different because its gametocytes take longer to develop – about 12 days to mature from a young parasite (merozoite) – and the mature gametocytes may remain infective in the peripheral circulation for up to several weeks after the patient has



\* When parasites are sensitive to the drug unless otherwise stated. Positive and negative arrows indicate the effect of the drug, enhancement (+) and suppression (-) respectively, on the parasite stage or its development.

Figure A4.2 Transmission of *Plasmodium falciparum* and the effects of antimalarials

been successfully treated for the asexual blood infection. In order to terminate infectivity of *P. falciparum*, the infection needs to be treated with drugs that have specific activity against gametocytes, i.e. either ACTs that destroy immature gametocytes, or by the addition of primaquine to the treatment regime to eradicate mature gametocytes. It is not known whether the use of primaquine with ACTs would result in a further suppression of infectivity, although it appears possible in principle, given that the two drugs act on different developmental stages of gametocytes.

### A4.3.2 The effect on transmission of using transmission-blocking medicines

The most direct consequences of lowering the infectivity of patients by the use of drugs are to be seen in areas of low transmission, where symptomatic patients constitute the majority of the infectious reservoir. Here, a strategy to shorten the period of infectivity of patients, if it could be achieved on a wide scale, would have a significant impact on the parasite reservoir of infection and, therefore, on malaria transmission. A reduction in transmission would, in these situations, result in an almost proportionate reduction in the prevalence of infection and incidence of disease.

In areas of low to moderate transmission, therefore, the provision of prompt and effective treatment to malaria patients is important both as a means of achieving the public health goal of reducing transmission, and attaining the therapeutic goal of reducing morbidity. Also important in these situations is the use of specific gametocytocidal drugs. There are anecdotal accounts from areas of low transmission with inadequate health services that offer little access to treatment, of patients presenting with extremely high parasite prevalence rates (>70%), approaching those found in areas of intense transmission (Figure A4.1). When treatment centres were established in such areas and early and effective malaria treatment was provided to patients, parasite prevalence and disease incidence rates decreased dramatically. One well-documented example is from the north-western border of Thailand where high incidence rates of *P. falciparum* prevailed in the face of increasing resistance to mefloquine, the antimalarial in use at the time. There, the deployment of artesunate in combination with mefloquine led to a significant decline in the incidence of the disease (12).

In high-transmission settings, infected but asymptomatic persons constitute an important part of the infectious reservoir. Even though treated cases (mainly children) have higher densities of gametocytes, and effectiveness of transmission

is positively related to gametocyte density, a treatment strategy to reduce infectivity of patients whose contribution to the reservoir of infection is only partial is not likely to have a major impact on transmission. This, together with the fact that a much greater reduction in transmission rates needs to be achieved in order to reduce parasite prevalence (and incidence of disease), makes the case for introducing an infectivity-suppressing component to the drug treatment of patients less compelling as a strategy for reducing the incidence of disease. However, the potentially important role of medication in reducing transmission must not be overlooked even in these situations. As intensified malaria control efforts deploying highly effective interventions, such as use of insecticide-treated mosquito nets and indoor residual spraying with insecticides, get under way, malaria inoculation rates could fall considerably (13). Transmission-reducing drug regimes will then have a greater role to play, and will complement other methods to achieve an impact on mortality and the incidence of malaria.

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## A4.4 Dynamics of drug pressure and transmission of drug-resistant genes

### A4.4.1 The continued use of a failing drug will confer a selective transmission advantage to resistant parasites

It has been shown that when resistance to a drug is prevalent in a locality, the continued use of that drug will confer a selective advantage to parasites carrying resistance genes, and will lead to higher rates of transmission of drug-resistant parasites. This will result in the rapid spread of the drug resistance through two mechanisms. First the use of the drug leads to higher numbers of circulating gametocytes in the resistant infections than in the sensitive ones (5, 6, 9, 10, 14, 15). The failing drug may reduce asexual parasitaemias initially to an extent that they may be undetectable even by PCR, but it induces the production of detectable numbers of gametocytes carrying the resistant genes. Resistance is associated with recrudescence. The subsequent recrudescence is associated with higher rates of gametocyte carriage than the primary infection. The recrudescence with resistance parasites is also more likely to fail treatment and recrudescence again than the primary infection. Thus cumulatively the resistant infection generates more gametocytes than an infection with sensitive parasites. Secondly, gametocytes carrying resistant genes

have been shown to be more infectious to mosquitoes. They produce higher densities of parasites (oocysts) in the mosquitoes, and infect a higher proportion of mosquitoes than those carrying sensitive genes (6, 7, 11). Molecular studies on the transmission of two *P. falciparum* genes linked to chloroquine resistance, *pfcr* and *pfmdr*, showed that gametocytes carrying the former produced more oocysts and were also more infectious to mosquitoes than gametocytes of the sensitive genotype (15).

#### A4.4.2 Reversal of transmission advantage by artemisinins

The use of drugs in combination, specifically with artemisinin derivatives, will remove the survival advantage conferred on parasites resistant to a particular drug by the use of that drug as monotherapy (10, 15, 16). This is because artemisinins are very effective in clearing blood parasites and also in reducing gametocyte prevalence and density (10), and therefore infectivity. But high cure rates are needed to prevent recrudescence with its greater carriage, and so it is inadvisable to combine an artemisinin derivative with a failing partner drug. Artemisinins have a short *in vivo* half-life so that their gametocytocidal activity will soon cease, leaving the parasites exposed to the failing partner drug, which has a longer *in vivo* half-life. There is a high failure rate and transmission of resistance parasites is not prevented (9, 10, 15). The clear advantage of using artemisinins in combination with an effective partners drug is that it will delay the selection and spread of drug-resistant genes (10–12, 15, 16).

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## A4.5 The role of transmission-blocking interventions and antimalarials in curtailing the spread of drug resistance

### A4.5.1 Transmission control

A reduction in transmission will curtail the spread of parasites of both sensitive and resistant strains. However, there is evidence to suggest that, in the absence of drug pressure, resistant parasites are at a survival disadvantage compared to sensitive strains, i.e. in the absence of selection by the drug to which they are resistant, drug-resistant parasites tend to be intrinsically less able to be transmitted than are drug-sensitive parasites (17, 18). These more stringent transmission conditions will, therefore, tend to selectively eliminate drug-resistant parasites (19). This expectation is supported by observations in Zimbabwe, where house spraying with insecticides to reduce malaria transmission was associated with reductions in the amount of drug resistance in the malaria parasites (20). Likewise, in low-transmission settings in India and Sri Lanka, the replacement of the failing chloroquine with an effective antimalarial, in combination with intense entomological transmission control, led to significant reductions, and in some instances even elimination, of chloroquine-resistant *P. falciparum*. In western Thailand where, in the 1990s, increasing levels of mefloquine resistance were associated with rising malaria incidence, the deployment of combination therapy for malaria treatment with mefloquine and artesunate was associated with an increased *in vitro* susceptibility of *P. falciparum* to mefloquine (12).

### A4.5.2 Antimalarials

In contrast to transmission control methods, such as residual insecticide spraying and the use of insecticide-treated nets – which are constantly in effect against the entire parasite population through the killing of the vector mosquito and the prevention of biting – treatment with antimalarials affects only the parasites in an infected person at the time of treatment. In high-transmission situations, this is a relatively rare event because the proportion of persons who are ill among those who are infected is quite small, and applies only to a small fraction of the parasite population. Therefore, the impact of drug treatment as a means of curtailing the spread of resistant parasites may be small in comparison to that of vector control methods.

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## A4.6 Conclusion

In situations of low malaria transmission, antimalarials have been and are being used for the specific purpose of reducing infectivity to mosquitoes – a notable example being the use of primaquine in the treatment of *P. falciparum* malaria. In areas of intense transmission, however, suppression of parasite infectivity has previously not been regarded as a significant goal of treatment. Now, the situation has changed. Artemisinin derivatives (which are gametocytocidal as well as destroying asexual stages of the parasite) are being widely deployed for the treatment of malarial disease, including in areas of intense transmission. This will allow the impact of anti-infective drugs on the reservoir of infection and transmission rates to be evaluated across the entire range of transmission intensities.

Reducing transmission is fundamental to the curtailment of drug resistance, and antimalarials can help achieve this, at least in some situations. This has implications for malaria treatment policy and also for drug development. The ability to suppress parasite infectivity should be included in the product profile of compounds that are being evaluated as potential new antimalarials.

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## ANNEX 5

### MALARIA DIAGNOSIS

#### A5.1 Symptom-based (clinical) diagnosis

The signs and symptoms of malaria, such as fever, chills, headache and anorexia, are non-specific and are common to many diseases and conditions. Malaria is a common cause of fever and illness in endemic areas (1, 2), *but* it is not possible to apply any one set of clinical criteria to the diagnosis of all types of malaria in all patient populations. The appropriateness of particular clinical diagnostic criteria varies from area to area according to the intensity of transmission, the species of malaria parasite, other prevailing causes of fever, and the health service infrastructure (3). One of the factors leading to a change in the clinical epidemiology of malaria in some areas is the prevalence of HIV/AIDS. This disease can increase the risk of acquiring malaria or the progression to severe malaria, depending on malaria transmission in the area and the age of the patient. The prevalence of HIV/AIDS can also lead to an increase in the incidence of febrile disease that is not malaria, and can therefore cause further difficulties in the symptom-based diagnosis of malaria (4).

Two different studies in The Gambia have shown that a sensitivity of 70–88% and a specificity of 63–82% for malaria diagnosis could be achieved using a weighting and scoring system for clinical signs and symptoms. These methods may be too complicated to implement and supervise under operational conditions in the field, and many of the key symptoms and signs of malaria in one area may not be applicable elsewhere. For instance, reduced feeding in a child is more likely to indicate malaria in The Gambia than in Ethiopia (5, 6).

Fever alone is as effective a criterion for diagnosis as clinical algorithms; a review of 10 studies indicated that use of the more restrictive criteria of clinical algorithms resulted in only trivial savings in drug costs compared with use of a fever-based diagnosis, even in areas of low malaria prevalence. In areas of high prevalence it greatly increases the probability of missing malaria infections (7).

#### A5.2 Light microscopy

In addition to providing a diagnosis with a high degree of sensitivity and specificity when performed well, microscopy allows quantification of malaria parasites and identification of the infecting species. It is inexpensive, the cost varying from US\$ 0.40–0.70 per slide and is considered to be the “gold standard” against which the sensitivity and specificity of other methods must

be assessed. A skilled microscopist is able to detect asexual parasites at densities of fewer than 10 per  $\mu\text{l}$  of blood but under typical field conditions the limit of sensitivity is approximately 100 parasites per  $\mu\text{l}$  (8). Light microscopy has important advantages:

- low direct costs if the infrastructure to maintain the service is available,
- high sensitivity if the quality of microscopy is high,
- differentiation between plasmodia species,
- determination of parasite densities,
- can be used to diagnose many other conditions.

It can be difficult to maintain good quality of microscopy, for various reasons: the need for adequate training and supervision of laboratory staff; the need to rely on electricity at night time; delays in providing results to patients; and the need for maintaining quality assurance and control of laboratory services.

Numerous attempts have been made to improve malaria microscopy, but none has proven superior to the classical method of Giemsa-staining and oil-immersion microscopy for performance in typical health-care settings (9).

### A5.3 Rapid diagnostic tests

Rapid diagnostic tests (RDTs) are immunochromatographic tests that detect parasite-specific antigens in a finger-prick blood sample. Some tests detect only one species (*Plasmodium falciparum*), others detect one or more of the other three species of human malaria parasites (*P. vivax*, *P. malariae* and *P. ovale*) (10–12). RDTs are available commercially in different formats, as dipsticks, cassettes or cards. Cassettes and cards are easier to use in difficult conditions outside health facilities.

RDTs are simple to perform and interpret, and do not require electricity or special equipment. WHO recommends that such tests should have a sensitivity of > 95% in detecting plasmodia at densities of more than 100 parasites per  $\mu\text{l}$  of blood. Programme and project managers should make their own choice among the many products available, using the criteria recommended by WHO ([www.wpro.who.int/rdt](http://www.wpro.who.int/rdt)) as there is as yet no international mechanism for pre-qualification of RDTs.

Current tests are based on the detection of histidine-rich protein 2 (HRP2), which is specific for *P. falciparum*, pan-specific or species-specific parasite lactate dehydrogenase (pLDH), or other pan-specific antigens such as aldolase. These antigens have different characteristics, which may affect suitability

for use in different situations, and this should be taken into account when developing RDT policy. These tests have many potential advantages, including:

- the ability to provide rapid results,
- fewer requirements for training and skilled personnel (a general health worker can be trained in one day),
- reinforcement of patient confidence in the diagnosis and in the health service in general.

There are also potential disadvantages, including:

- the likelihood of misinterpreting a positive result as indicating malaria in patients with parasitaemia incidental to another illness, in particular when host immunity is high; the inability in the case of some RDTs, to distinguish new infections from a recently and effectively treated infection; this is due to the persistence of certain target antigens (e.g. HRP2) in the blood for 1–3 weeks after effective treatment. The persistence of PfHRP2 in blood for at least one week after treatment can be used in the diagnosis of severe malaria in low transmission areas where artemisinin derivatives are widely available. Patients may have cleared peripheral parasitaemia because of inadequate self treatment, but the PfHRP2 test will be strongly positive.
- unpredictable sensitivity in the field (13–20), mainly because test performance is greatly affected by adverse environmental conditions such as high temperature and humidity.

Published sensitivities of RDTs for *P. falciparum* range from comparable to those of good field microscopy (>90% at 100–500 parasites/ $\mu$ l of blood) to very poor (40–50%) for some widely used products. Sensitivities are generally lower for other species. The reasons for poor sensitivity are not clear. They may include: poor test manufacture, damage due to exposure to high temperature or humidity, incorrect handling by end-users, possible geographical variation in the test antigen, and poor comparative microscopy (12). Several studies have shown that health workers, volunteers and private sector providers can, with some support and follow-up, learn to use RDTs correctly with relative ease.

The use of a confirmatory diagnosis with either microscopy or RDTs is expected to reduce the overuse of antimalarials by ensuring that treatment is targeted on patients with confirmed malaria infections as opposed to treating all patients with fever. There is, however, little documented evidence that this is so. The main problem is that providers of care, although they may be willing to perform diagnostic tests, do not always comply with the results, especially when they are negative. Being aware that delay in providing effective treatment can be fatal for a malaria patient, they are often reluctant to withhold treatment on the basis of a negative result. WHO is currently supporting operational research projects designed to address these issues.

## A5.4 Immunodiagnosis and PCR-based molecular detection methods

Detection of antibodies to parasites, which may be useful for epidemiological studies, is neither sensitive, specific, nor rapid enough to be of use in the management of patients suspected of having malaria (21).

Techniques to detect parasite DNA based on the polymerase chain reaction (PCR) are highly sensitive and very useful for detecting mixed infections, in particular at low parasite densities. They are also useful for studies on drug resistance and other specialized epidemiological investigations (22), but are not generally available in malaria endemic areas.

### **Interventions: use of RDTs or microscopy for diagnosis**

*Summary of RCTs:* despite the existence of a number of studies on the sensitivity and specificity of various methods for diagnosing malaria, there are no RCTs on the impact of a confirmatory diagnosis as an intervention.

*Expert comment:* treatment of all people with fever leads to overuse of antimalarials in most settings. With the introduction of more expensive antimalarials, there is a need to target treatment more effectively on people with malaria infections by using confirmatory testing. This will have the additional benefits of improving patient care and providing better epidemiological surveillance data. However, in young children in areas of intense transmission, it is believed that the risk associated with relying on a parasitological diagnosis (death owing to a false negative result) may outweigh the benefits.

except for children in areas of intense transmission, confirmatory (parasitological) testing should be introduced and used to supplement clinical criteria in diagnosis.

Controlled trials and operational research in various settings are urgently needed.

<sup>a</sup> See also references (7) and (23).

## A5.5 References

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## ANNEX 6

# RESISTANCE TO ANTIMALARIALS

### A6.1 Introduction

There are currently no bedside tests for determining the susceptibility of the malaria parasite to antimalarials. Monitoring is therefore needed to determine geographical trends in susceptibility and the emergence and spread of drug resistance. The information obtained will help guide treatment choices and predictions about future resistance patterns.

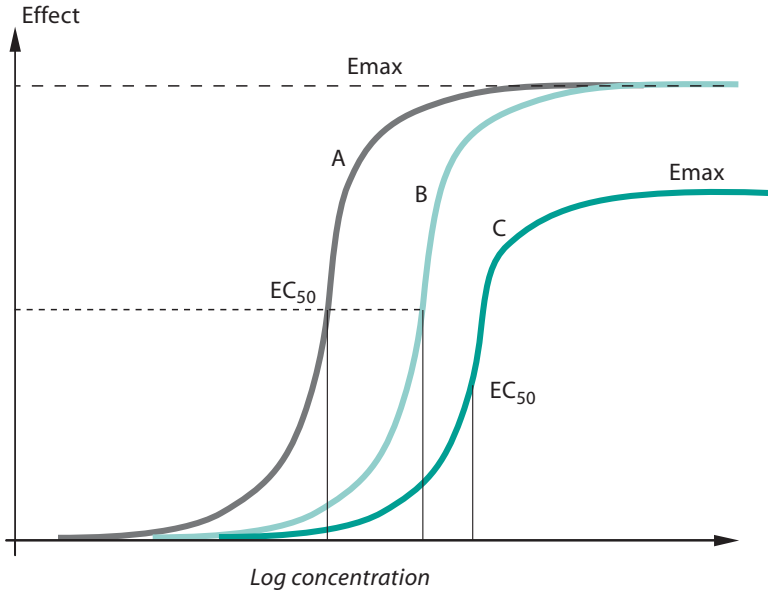
The greatest problem with drug resistance occur with *P. falciparum*. Resistance of *P. falciparum* is of particular concern because of the enormous burden of disease caused by this species, its lethal potential, the propensity for epidemics, and the cost of candidate replacement drugs for areas with established drug resistance. Chloroquine resistance does occur in *P. vivax*, especially in western Oceania, but there are very few reports of resistance in *P. malariae* or *P. ovale* (although there have also been very few studies).

This annex defines resistance, examines how it arises and spreads, summarizes its current global distribution and describes ways in which it can be monitored.

### A6.2 Definition

Antimalarial drug resistance is defined as the ability of a parasite strain to survive and/or multiply despite the proper administration and absorption of an antimalarial drug in the dose normally recommended. Drug resistance to an antimalarial compound results in a right shift in the concentration–effect (dose–response) relationship (Figure A6.1). As the pharmacokinetic properties of antimalarials vary widely in different individuals, the definition of resistance should probably also include a “normal” plasma concentration profile for the active drug concerned or, in the case of a prodrug (a drug that is not active in the ingested form and requires chemical conversion through metabolic processes to become pharmacologically active), a “normal” profile of the biologically active metabolite. Antimalarial drug resistance is not necessarily the same as malaria “treatment failure”, which is a failure to clear malarial parasitaemia and/or resolve clinical symptoms despite the administration of an antimalarial. So while drug resistance may lead to treatment failure, not all treatment failures are caused by drug resistance. Treatment failure can also be the result of incorrect dosing, problems of treatment adherence (compliance),

poor drug quality, interactions with other drugs, compromised drug absorption, or misdiagnosis of the patient. Apart from leading to inappropriate case management, all these factors may also accelerate the spread of true drug resistance by exposure of the parasites to inadequate drug levels.



**Figure A6.1** Resistance is a rightward shift in the concentration–effect relationship for a particular parasite population. This may be a parallel shift (B) from the “normal” profile (A) or, in some circumstances, the slope changes, and/or the maximum achievable effect is reduced (C). The effect is parasite killing

### A6.3 The emergence and spread of antimalarial resistance

The development of resistance can be considered in two parts: the initial genetic event, which produces the resistant mutant; and the subsequent selection process in which the survival advantage in the presence of the drug leads to preferential transmission of resistant mutants and thus the spread of resistance. In the absence of the antimalarial, resistant mutants may have a survival disadvantage. This “fitness cost” of the resistance mechanism may result in a decline in the prevalence of resistance once drug pressure is removed.

Resistance to one drug may select for resistance to another where the mechanisms of resistance are similar (cross-resistance). There are many parallels with

antibiotic resistance, in particular resistance to antituberculosis drugs where, as for malaria, transferable resistance genes are not involved in the emergence of resistance (1–3). In experimental models, drug-resistant mutations can be selected without mosquito passage (i.e. without meiotic recombination) by exposure of large numbers of malaria parasites (either *in vitro*, in animals, or as was done in the past, in volunteers) to subtherapeutic drug concentrations (4).

Various factors determine the propensity for antimalarial drug resistance to develop (5):

- the intrinsic frequency with which the genetic changes occur,
- the degree of resistance (the shift in the concentration-effect relationship, (Figure A6.1) conferred by the genetic change,
- the fitness cost of the resistance mechanism,
- the proportion of all transmissible infections that are exposed to the drug (the selection pressure),
- the number of parasites exposed to the drug,
- the concentrations of drug to which these parasites are exposed ,
- the pharmacokinetic and pharmacodynamic properties of the antimalarial,
- individual (dosing, duration, adherence) and community (quality, availability, distribution) patterns of drug use,
- the immunity profile of the community and the individual,
- the simultaneous presence of other antimalarials or substances in the blood to which the parasite is not resistant.

The emergence of resistance can be thought of in terms of the product of the probabilities of *de novo* emergence (a rare event) and subsequent spread. Resistant parasites, if present, will be selected when parasites are exposed to “selective” (subtherapeutic) drug concentrations. “Selective” in this context means a concentration of drug that will eradicate the sensitive parasites but still allow growth of the resistant parasite population such that it eventually transmits to another person. Because *de novo* resistance arises randomly among malaria parasites, non-immune patients infected with large numbers of parasites who receive inadequate treatment (either because of poor drug quality, poor adherence, vomiting of an oral treatment, etc.) are a potent source of *de novo* resistance. This emphasizes the importance of correct prescribing, and good adherence to prescribed drug regimens, and also provision of treatment regimens that are still highly effective in hyperparasitaemic patients. The principle specific immune response that controls the primary symptomatic infection in falciparum malaria is directed by the variant surface antigen (PfEMP1). The parasite population evades this immune response by switching its surface antigen in a specific sequence of changes. The probability

of selecting a resistant parasite from the primary infection is the product of the switch rate and the rate of formation of viable resistant parasites.

The subsequent spread of resistant mutant malaria parasites is facilitated by the widespread use of drugs with long elimination phases. These provide a “selective filter”, allowing infection by the resistant parasites while the residual antimalarial activity prevents infection by sensitive parasites. Slowly eliminated drugs such as mefloquine (terminal elimination half-life ( $T_{1/2\beta}$ ) 2–3 weeks) or chloroquine ( $T_{1/2\beta}$  1–2 months) persist in the blood and provide a selective filter for months after drug administration has ceased.

### A6.3.1 Transmission intensity and the selection and spread of resistance

The recrudescence and subsequent transmission of an infection that has generated a *de novo* resistant malaria parasite is essential for resistance to be propagated (5). Gametocytes carrying the resistance genes will not reach transmissible densities until the resistant biomass has expanded to numbers close to those producing illness ( $> 10^7$  parasites) (6). Thus to prevent resistance spreading from an infection that has generated *de novo* resistance, gametocyte production from the recrudescence resistant infection must be prevented. There has been debate as to whether resistance arises more rapidly in low- or high-transmission settings (7, 8), but aside from theoretical calculations, epidemiological studies clearly implicate low-transmission settings as the source of drug resistance. Chloroquine resistance and high-level sulfadoxine-pyrimethamine resistance in *P. falciparum* both originated in South-East Asia and subsequently spread to Africa (9).

In low-transmission areas, the majority of malaria infections are symptomatic and selection therefore takes place in the context of treatment. Relatively large numbers of parasites in an individual usually encounter antimalarials at concentrations that are maximally effective. But in a variable proportion of patients, for the reasons mentioned earlier, blood concentrations are low and may select for resistance.

In high-transmission areas, the majority of infections are asymptomatic and infections are acquired repeatedly throughout life. Symptomatic and sometimes fatal malaria occurs in the first years of life, but thereafter it is increasingly likely to be asymptomatic. This reflects a state of imperfect immunity (premunition), where the infection is controlled, usually at levels below those causing symptoms. The rate at which premunition is acquired depends on the intensity of transmission. In the context of intense malaria transmission, people still receive antimalarial treatments throughout their lives (often inappropriately for

other febrile infections), but these “treatments” are largely unrelated to the peaks of parasitaemia, thereby reducing the probability of selection for resistance.

Immunity considerably reduces the emergence of resistance (9). Host defence contributes a major antiparasitic effect, and any spontaneously generated drug-resistant mutant malaria parasite must contend not only with the concentrations of antimalarial present, but also with host immunity. This kills parasites regardless of their antimalarial resistance, and reduces the probability of parasite survival (independently of drugs) at all stages of the transmission cycle. For the blood stage infection, immunity acts in a similar way to antimalarials both to eliminate the rare *de novo* resistant mutants and stop them being transmitted (i.e. like a combination therapy), and also to improve cure rates with failing drugs (i.e. drugs falling to resistance) thereby reducing the relative transmission advantage of resistant parasites. Even if a resistant mutant does survive the initial drug treatment and multiplies, the chance that this will result in sufficient gametocytes for transmission is reduced as a result of asexual stage immunity (which reduces the multiplication rate and lowers the density at which the infection is controlled) and transmission-blocking immunity. Furthermore, other parasite genotypes are likely to be present, competing with the resistant parasites for red cells, and increasing the possibility of outbreeding of multigenic resistance mechanisms or competition in the feeding anopheline mosquito (10).

### A6.3.2 Antimalarial pharmacodynamics and the selection of resistance

The genetic events that confer antimalarial drug resistance (while retaining parasite viability) are spontaneous and rare. They are thought to be independent of the drug. The resistance mechanisms that have been described are mutations in genes or changes in the copy number of genes relating to the drug’s target or pumps that affect intraparasitic concentrations of the drug. A single genetic event may be all that is required, or multiple unlinked events may be necessary (epistasis). *P. falciparum* parasites from South-East Asia seem constitutionally to have an increased propensity to develop drug resistance.

#### Aminoquinolines

Chloroquine resistance in *P. falciparum* may be multigenic and is initially conferred by mutations in a gene that encodes a transporter (PfCRT). PfCRT may be an anion channel pumping chloroquine out from the food vacuole. The initial mutation, which confers a moderate level of chloroquine resistance, is replacement of a lysine with threonine at codon 76. Positions 72 to 76 are critical for the binding of desethylamodiaquine (the biologically active metabolite of

amodiaquine) and also verapamil (which may reverse chloroquine resistance *in vitro*). Eleven other PfCRT mutations have been described to date. These additional mutations may contribute to aminoquinoline resistance, although the precise mechanisms have not yet been determined. Amodiaquine resistance is linked to chloroquine resistance, but is not well characterized. In the presence of PfCRT mutations, point mutations in a second transporter (PfMDR1) modulate the level of *P. falciparum* resistance *in vitro*. Parasites that are highly resistant to chloroquine often have Lys76Thr and Ala220Ser in PfCRT, and Asn86Tyr in PfMDR. The role of PfMDR1 mutations in determining the therapeutic response following chloroquine treatment is still unclear. The cause of chloroquine resistance in *P. vivax* has not been found yet.

### Mefloquine

Resistance to mefloquine and other structurally related aryl-amino-alcohols in *P. falciparum* results from amplifications (i.e. duplications not mutations) in *Pfmdr*, which encodes an energy-demanding *p*-glycoprotein pump. This explains approximately two-thirds of the variance in susceptibility. Interestingly, it appears that generally only the “wild type” (PfMDR Asn86) amplifies, so that in the transition from chloroquine resistance, back mutation from mutant to wild type precedes amplification. Gene duplication is particularly frequent in the *P. falciparum* genome. It is a much more common genetic event than mutation. The low background frequency of gene amplification suggests that it may well confer a fitness disadvantage in the absence of selective pressure.

The products of these various genetic events result in reduced intracellular concentrations of the antimalarial quinolines in the parasite (the relative importance of reduced uptake and increased efflux remains unresolved).

### Antifolate antimalarials

For the antifolate antimalarials (pyrimethamine, and the biguanides cycloguanil and chlorcycloguanil – the active metabolites of proguanil and chlorproguanil, respectively) resistance in *P. falciparum* and *P. vivax* results from the sequential acquisition of mutations in the gene (*dhfr*) that encodes dihydrofolate reductase (DHFR). Each mutation confers a stepwise reduction in susceptibility. In *P. falciparum*, the initial mutation is almost invariably at position 108 (usually serine to asparagine), which confers only a ten-fold reduction in drug susceptibility, and does not affect therapeutic responses to sulfadoxine-pyrimethamine. This has little clinical relevance initially, but then mutations arise at positions 51 and 59, conferring increasing resistance to pyrimethamine containing medicines. Infections with triple mutants are relatively resistant but some therapeutic response is usually seen. The acquisition of a fourth and devastating mutation at position 164 (isoleucine to leucine) renders the

available antifolates completely ineffective (11). Interestingly, mutations conferring moderate pyrimethamine resistance do not necessarily confer cycloguanil resistance, and vice versa. For example, mutations at positions 16 (alanine to valine) plus 108 (serine to threonine) confer high-level resistance to cycloguanil but not to pyrimethamine. In general, the biguanides are more active than pyrimethamine against the resistant mutants (and they are more effective clinically too), but they are ineffective against parasites with the DHFR mutation at position 164. *P. vivax* shares similar antifolate resistance mechanisms through serial acquisition of mutations in PvDHFR. The sequence of acquisition associated with increasing resistance is usually mutation at position 117 or 58, followed by mutation at positions 57, 61 and then 13.

### Sulfonamide and sulfone

The marked synergy with sulfonamides and sulfones is very important for the antimalarial activity of sulfa-pyrimethamine or sulfone-biguanide combinations. In *P. falciparum*, sulfonamide and sulfone resistance also develops by progressive acquisition of mutations in the gene encoding the target enzyme PfDHPS (which is a bifunctional protein with the enzyme PPPK). Specifically altered amino acid residues have been found at positions 436, 437, 540, 581 and 613 in the PfDHPS domain. The mutations at positions 581 and 631 do not occur in isolation, but always following an initial mutation (usually at position 437, alanine to glycine). Mutations in *P. vivax* DHPS (at positions 383 and 553) also appear to contribute to resistance.

### Atovaquone-proguanil

Resistance to atovaquone results from point mutations in the gene (*cytB*), which encodes cytochrome b. In the atovaquone-proguanil combination, it is proguanil itself probably acting on the mitochondrial membrane rather than the *dhfr*-inhibiting proguanil metabolite cycloguanil that appears to be important in this combination. Whether and how resistance develops to the mitochondrial action of proguanil is not known.

### Artemisinin

Although a target for the artemisinins has recently been identified (PfATPase6), preliminary studies have not so far associated polymorphisms in the gene encoding this enzyme with reduced susceptibility of malaria parasites. Amplification in PfMDR does reduce artemisinin susceptibility *in vitro*, but not to a degree that causes *in vivo* resistance. This has led to erroneous claims that artemisinin resistance was being selected by widespread use of artemisinin derivatives, whereas in fact the selection pressure came from mefloquine use.

The mutation frequencies derived from *in vitro* studies are often much higher than those derived from observations *in vivo* (12). The absence of host defences and differences in antimalarial concentration profiles contribute to this discrepancy. The highest rates of emergence of resistance *in vivo* are for pyrimethamine and atovaquone. In the case of atovaquone, it has been estimated that one in three patients with symptomatic falciparum “contained”, at presentation, a spontaneously arising atovaquone-resistant mutant parasite (5). For drugs such as chloroquine or artemisinin, the genetic events conferring resistance are much rarer. These genetic events may result in moderate changes in drug susceptibility, such that the drug still remains effective (e.g. as in the 108AsnDHFR mutation for pyrimethamine resistance) or, less commonly, very large reductions in susceptibility such that achievable concentrations of the drug are completely ineffective (e.g. as the cytochrome B mutations giving rise to atovaquone resistance) (13–16).

### A6.3.3 Antimalarial pharmacokinetics and the selection of resistance

#### *Absorption and disposition*

The probability of selecting a *de novo* mutation that is resistant to antimalarials during the initial phase of treatment depends on the per-parasite frequency of the genetic event, the number of parasites present, immunity in the infected individual, and the relationship between the drug levels achieved and the degree of resistance conferred by the mutant parasite. Obviously, if the range of blood concentrations achieved in the patient considerably exceeds the concentrations giving 90% inhibition of multiplication ( $IC_{90}$  values) for the most resistant mutant ( $IC_{90}^R$ ), then resistance cannot be selected in the acute phase of treatment as even the resistant mutants are prevented from multiplying. Conversely, if the degree of resistance provided by the genetic event is very small, the window of opportunity for selection may be negligible. Provided that there is such a window of selection then the broader the range of peak antimalarial concentrations and the closer the median value approaches  $IC_{90}^R$ , the greater the probability of selecting a resistant mutant in a patient. Peak drug concentrations are determined by absorption, distribution volume and dose. Several antimalarials (notably lumefantrine, halofantrine, atovaquone and, to a lesser extent, mefloquine) are lipophilic, hydrophobic and very variably absorbed (interindividual variation in bioavailability up to 20-fold) (17, 18). Interindividual variation in distribution volumes tends to be lower (usually less than five-fold) but, taken together with variable absorption, the outcome is considerable interindividual variation in peak antimalarial blood concentrations. The main sources of underdosing globally are incorrect self-medication because of poor

adherence to the correctly prescribed drug regimen, poor quality drugs, uncontrolled drug availability and purchase of incorrect dose regimens, use of substandard drugs purchased in shops or markets, and incorrect administration in the home. The acute infection is the principal source of *de novo* resistance selection. Quality assured drugs, education, correct prescribing, good adherence, and optimized packaging and formulations therefore play pivotal roles in preventing the emergence of antimalarial drug resistance.

### *Drug elimination rates*

In some areas of the world, transmission intensities may be as high as three infectious bites per person per day. In this context, a person who takes antimalarial treatment for symptomatic malaria exposes not only the parasites causing that infection to the drug, but also any newly acquired infections that emerge from the liver during the drug's elimination phase; the longer the terminal elimination half-life, the greater the exposure. The length of the terminal elimination half-life is an important determinant of the propensity for an antimalarial to select for resistance (19–21). Some rapidly eliminated antimalarials (e.g. the artemisinin derivatives) never present an intermediate drug concentration to infecting malaria parasites because they are eliminated completely within the two-day life-cycle of the asexual parasite. Others (e.g. mefloquine, chloroquine) have elimination half-lives of weeks or months and present a lengthy selection opportunity.

With the exception of the artemisinin derivatives, maximum antimalarial parasite reduction ratios (kill rates) do not exceed 1000-fold per cycle (22). Following hepatic schizogony, exposure of at least two asexual cycles (4 days) to therapeutic drug concentrations is therefore required to eradicate the blood stage parasites emerging from the liver. Even with maximum kill rates in the sensitive parasites and maximum growth rates in the resistant parasites, the resistant parasites only “overtake” the sensitive parasites in the third asexual cycle. Thus rapidly eliminated drugs (such as the artemisinin derivatives or quinine) cannot select during the elimination phase. Obviously, the greater the degree of resistance conferred by the resistance mutation – i.e. the higher the  $IC_{90}^R$  relative to the  $IC_{90}$  for susceptible parasites ( $IC_{90}^S$ ) – the wider is the window of selection opportunity.

Patent gametocytaemia is more likely in recrudescence than in primary infections. Therefore, if *de novo* resistance arose in an acute symptomatic treated infection, the transmission probability from the subsequent recrudescence infection (bearing the new resistance genes) would be higher than from an infection newly acquired during the elimination phase of the antimalarial given for a previous infection, even if it attained the same parasite densities (23).

#### A6.3.4 Spread of resistance

Several mathematical models have been devised to examine the spread of antimalarial drug resistance (10, 21, 24, 25). Spread of resistance is determined by the reproductive advantage conferred by the resistance mechanism. This derives from the increased gametocyte carriage associated with treatment failure (both from the primary infection and the subsequent recrudescences) – the “donors”, and then the selective pressure from residual concentrations of slowly eliminated antimalarial in potential recipients. A long elimination half-life results in long periods of post-treatment chemoprophylaxis.

Resistance encoded by multiple mutations at a single locus may be considered in two overlapping phases. The first phase, in which the drug is better tolerated by the parasites but therapeutic doses still usually clear the infection, and the second phase, when clinical failures start to occur. This second phase is very rapid and it is essential that surveillance programmes are in place and capable of monitoring the change from the first to the second phase. In areas of high transmission, the first phase may occur faster, but the subsequent phase slower. Combination therapy significantly slows the rate of evolution of resistance, but it should be instigated before significant resistance to either component is present.

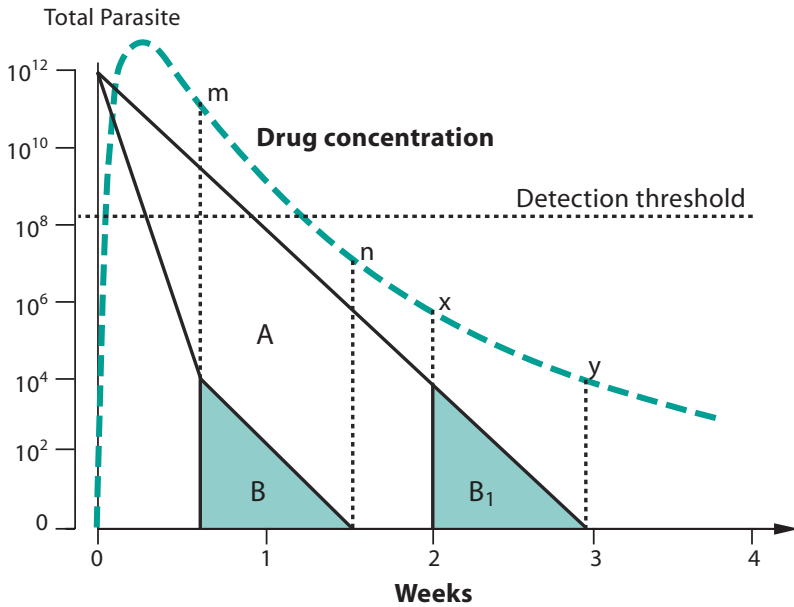
#### A6.3.5 Prevention of resistance by use of combination therapy

The theory underlying combination treatment of tuberculosis, leprosy and HIV infection is well known, and has recently been applied to malaria (4, 5, 24, 26–29). If two drugs with different modes of action, and therefore different resistance mechanisms, are used in combination, then the per-parasite probability of developing resistance to both drugs is the product of their individual per-parasite probabilities. For example, if the per-parasite probabilities of developing resistance to drug A and drug B are both 1 in  $10^{12}$ , then a simultaneously resistant mutant will arise spontaneously in 1 in  $10^{24}$  parasites. As it is postulated that there are approximately  $10^{17}$  parasites in the entire world, and a cumulative total of less than  $10^{20}$  in one year, such a simultaneously resistant parasite would arise spontaneously roughly once every 10 000 years – provided the drugs always confronted the parasites in combination. Thus the lower the *de novo* per-parasite probability of developing resistance, the greater the delay in the emergence of resistance.

Stable resistance to the artemisinin derivatives has not yet been identified, and cannot yet be induced in the laboratory, which suggests that it may be very rare indeed. *De novo* resistance to chloroquine is also very rare, and appears to have arisen and spread only twice in the world during the first decade of intensive use in the 1950s (30). On the other hand, resistance to antifolate and atovaquone

arises relatively frequently (e.g. antifolate resistance rose to high levels within two years of the initial deployment of proguanil in peninsular Malaya in 1947) and can be induced readily in experimental models (14, 27). Against a background of chloroquine resistance, mefloquine resistance arose over a six-year period on the north-west border of Thailand (31).

The ideal pharmacokinetic properties for an antimalarial have been much debated. Rapid elimination ensures that the residual concentrations do not provide a selective filter for resistant parasites, but drugs with this property (if used alone) must be given for at least 7 days, and adherence to 7-day regimens is poor. In order to be effective in a 3-day regimen, elimination half-lives usually need to exceed 24 h. Artemisinin derivatives are particularly effective in combinations with other antimalarials because of their very high killing rates (parasite reduction rate around 10 000-fold per cycle), lack of adverse effects and absence of significant resistance (5). Combinations of artemisinin derivatives (which are eliminated very rapidly) given for 3 days, with a slowly eliminated drug such as mefloquine, provide complete protection against the emergence of resistance to the artemisinin derivatives if adherence is good, but they do leave the slowly eliminated “tail” of mefloquine unprotected. Perhaps resistance could arise within the residual parasites that have not yet been killed by the artemisinin derivative. However, the number of parasites exposed to mefloquine alone is a tiny fraction (less than 0.00001%) of those present in the acute symptomatic infection. Furthermore, these residual parasites “see” relatively high levels of mefloquine and, even if susceptibility was reduced, these levels may be sufficient to eradicate the infection (Figure A6.2). The long mefloquine tail does, however, provide a selective filter for resistant parasites acquired from elsewhere, and therefore contributes to the spread of resistance once it has developed. Yet on the north-west border of Thailand, an area of low transmission where mefloquine resistance had already developed, systematic deployment of the artesunate-mefloquine combination was dramatically effective in stopping resistance and also in reducing the incidence of malaria (31, 32). This strategy is thought to be effective at preventing the emergence of resistance at higher levels of transmission, where high-biomass infections still constitutes the major source of *de novo* resistance.



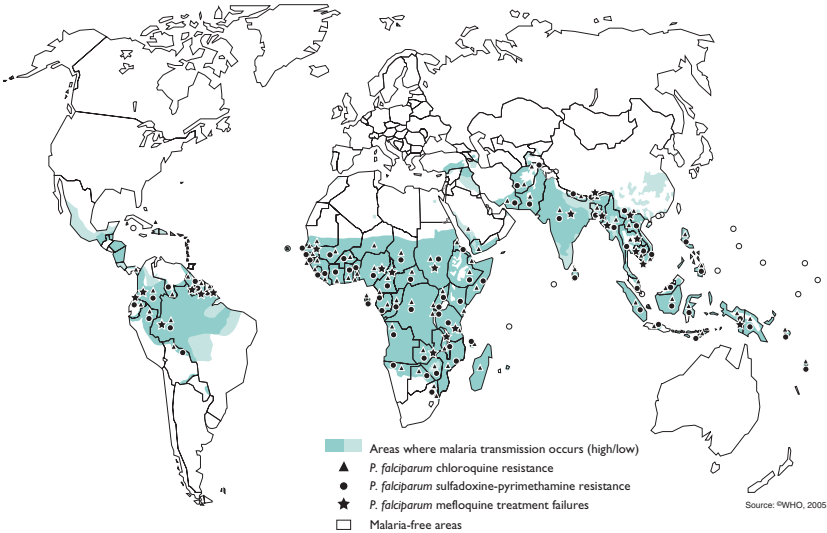
**Figure A6.2** The artesunate + mefloquine combination. If no artesunate is given, then the number of parasites exposed to mefloquine alone is given by the area of A; with the combination administered for 3 days, the number of parasites exposed to mefloquine alone is given by the area of B (100 million times fewer). Furthermore, mefloquine levels are higher (m to n) when confronting B than when confronting the same number of parasites ( $B_1$ ) if no artesunate is given (x to y). If a parasite containing a *de novo* mefloquine-resistant mutation were to occur, then such a parasite should still be susceptible to artesunate. Thus the probability of selecting a resistant mutant is reduced by 100 million times, as only a maximum of 100 000 parasites are exposed to mefloquine alone after the fourth day (i.e. in the third cycle), and any artesunate-resistant parasite selected by artesunate initially would always be killed by the accompanying mefloquine. As a result, the combination is more effective, reduces transmission and prevents the emergence of resistance to both drugs.

## A6.4 A summary of the global distribution of antimalarial drug resistance

Resistance to antimalarials has been a particular problem with *P. falciparum*, in which widespread resistance to chloroquine, sulfadoxine-pyrimethamine and mefloquine has been observed (Figure A6.3). Antifolate and chloroquine resistance has developed in *P. vivax* in several areas, and chloroquine resistance

in *P. malariae* has also recently been reported. No significant resistance has yet been observed to artemisinin and its derivatives despite their extensive deployment in several parts of Asia.

**Malaria transmission areas and reported *P. falciparum* resistance, 2004**

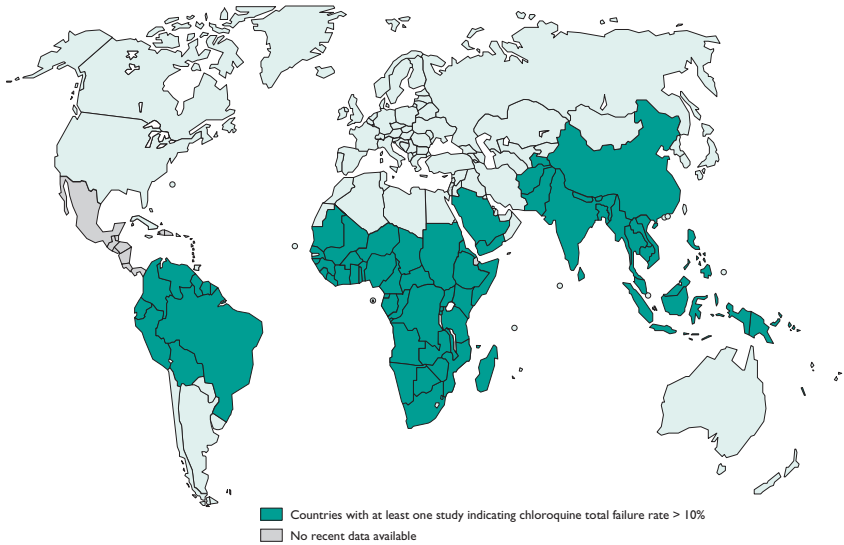


**Figure A6.3** Malaria transmission areas and the distribution of reported resistance or treatment failures with selected antimalarial drugs, September 2004 (mefloquine resistance in Africa is currently being further reviewed)

#### A6.4.1 *Plasmodium falciparum* resistance

##### Chloroquine

The first reports of chloroquine resistance occurred in Thailand and Colombia in the late 1950s, around 12 years after the drug's introduction. By 1980, all endemic areas in South America were affected, and by 1989, most of Asia and Oceania. In Africa, chloroquine resistance emerged in 1978 in the east, and gradually spread westwards through the 1980s. Resistance has now been documented in all *falciparum*-endemic areas except Central America and the Caribbean (33). Recent molecular studies favour importation of chloroquine resistance to Africa from East Asia (34, 35). Chloroquine resistance has emerged independently less than ten times in the past 50 years (Figure A6.4).

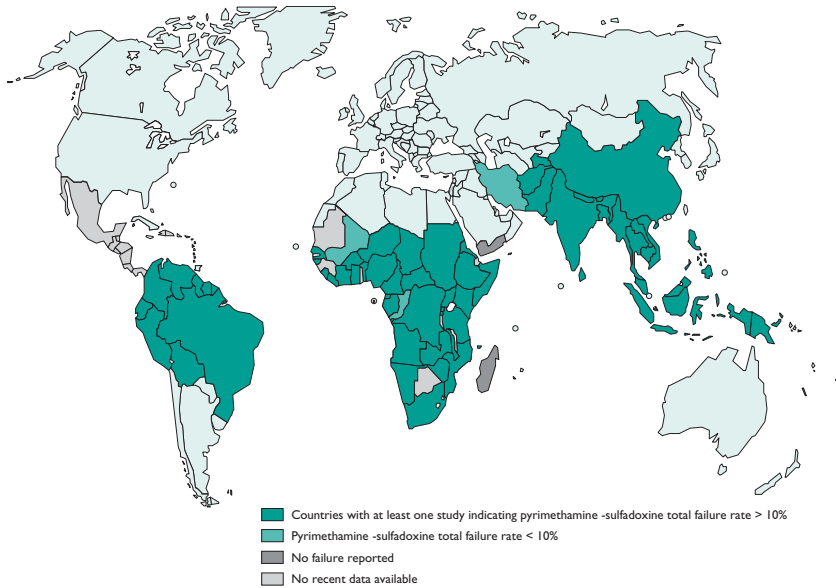


**Figure A6.4** Distribution of chloroquine resistance in *Plasmodium falciparum*

### Sulfadoxine-pyrimethamine

Resistance to pyrimethamine emerged rapidly after its deployment for treatment, prophylaxis and, in some areas, mass treatment in the 1950s. Resistance to both components of sulfadoxine-pyrimethamine was noted shortly after this drug was introduced over a decade later. In South-East Asia this occurred on the Thai-Cambodian border in the mid-1960s. Resistance became an operational problem in the same area within the few years of the introduction of sulfadoxine-pyrimethamine to the malaria control programme in 1975 (36). High-level resistance is found in many parts of South-East Asia, southern China and the Amazon basin, and lower levels of resistance are seen on the coast of South America and in southern Asia and Oceania. In eastern Africa, sulfadoxine-pyrimethamine sensitivity was observed to be declining in the 1980s and resistance has progressed westwards across Africa relentlessly over the last decade. Clinical failure rates of more than 25% have already been reported in Liberia (37), Guinea Bissau (38) and Malawi (39).

Many areas now have high-level resistance with high-treatment failure rates in children. Recent molecular evidence suggests a common South-East Asian origin of the resistant *P. falciparum* parasites now prevalent in much of southern and Central Africa (triple *dhfr* mutant) (9, 40–42) (Figure A6.5).

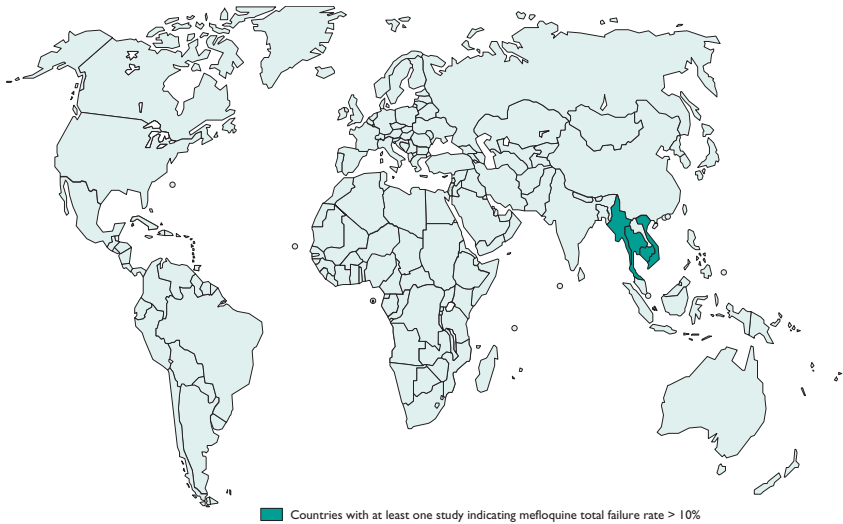


**Figure A6.5** Distribution of sulfadoxine-pyrimethamine resistance in *Plasmodium falciparum*

### Mefloquine

Mefloquine resistance was first observed on the Thai-Cambodian and Thai-Burmese borders in the late 1980s (43, 44) and the monotherapy is no longer effective there. Migrant gem miners returning from Cambodia may have been the means of spread of mefloquine resistance to India and Bangladesh (45). Isolated cases of mefloquine resistance have also been reported from the Amazon basin, and *in vitro* studies in Africa have identified some *P. falciparum* strains with low mefloquine sensitivity. Overall, clinical mefloquine resistance outside South-East Asia is rare (Figure A6.6).

The main determinant of mefloquine resistance is amplification of the gene (*Pfmdr*) that encodes the multidrug transporter (46). Amplification occurs only for the “wild type” allele explaining the inverse relationship between sensitivity to chloroquine (the *Pfmdr* Tyr86 mutation is associated with reduced sensitivity) and to mefloquine (and to the structurally related drugs, quinine and halofantrine) (15).



**Figure A6.6** Distribution of mefloquine resistance in *Plasmodium falciparum*

### Quinine

The first reports of possible quinine resistance occurred in Brazil almost 100 years ago. Even today, however, clinical resistance to quinine monotherapy is reported only sporadically in South-East Asia and western Oceania, and resistance in Africa and South America is much less frequent. Widespread use of quinine in Thailand in the 1980s led to significant reduction in its sensitivity (45). Quinine is therefore now used in combination with an antibiotic, usually tetracycline, doxycycline or clindamycin, and is reserved for cases of severe malaria. *Pfmdr1* mutations associated with chloroquine resistance have been believed to be associated with reduced susceptibility to quinine (47, 48).

### Artemisinin

Except in an animal model, there have been no confirmed reports of artemisinin resistance in malaria parasites that infect humans. The pharmacological characteristics of the drug, namely short elimination half-life, rapidity of action and ability to reduce gametocyte carriage, should delay the onset of significant resistance. Artemisinin derivatives are associated with high recrudescence rates (~10%) after monotherapy, so are usually combined with longer-acting antimalarials for clinical treatment. These recrudescences, however, are not a result of resistance.

### Multidrug resistance

Multidrug resistance is generally defined as resistance to three or more antimalarial compounds from different chemical classes. Generally, the first two classes are 4-aminoquinolines (e.g. chloroquine) and antifolates (e.g. sulfadoxine-pyrimethamine). The precise amount of resistance needed in order for a drug to be considered as failing is not universally agreed. Some consider clinical cure rates of less than 75% to be the minimum required for classification as failure, while the current recommendations aim for cure rates over 90%.

Established multidrug resistance occurs in South-East Asia (particularly along the borders of Thailand with Burma and Cambodia) and in the Amazon basin. In Thailand, mefloquine monotherapy was replaced with the combination of high-dose mefloquine and artesunate given for 3 days. Mefloquine resistance has been reduced by the use of this combination, as cure rates of more than 95% have been sustained for over 10 years, and susceptibility to mefloquine has actually improved despite extensive deployment of the combination.

Several areas are at risk of multidrug resistance, as resistance to chloroquine and sulfadoxine-pyrimethamine is already widespread. Progressive loss of sulfadoxine-pyrimethamine efficacy should be taken as a warning sign.

#### A6.4.2 *Plasmodium vivax* resistance

##### Chloroquine

Resistance of *P. vivax* is rare and generally limited to chloroquine resistance, which was first reported in the late 1980s in Papua New Guinea and Indonesia. Focal true chloroquine resistance (with whole blood chloroquine + desethylchloroquine concentrations of >100 ng/ml on the day of failure) or prophylactic and/or treatment failure not necessarily related to true resistance, have since also been observed in Brazil, Colombia, Ethiopia, Guatemala, Guyana, India, Republic of Korea, Myanmar, Solomon Islands, Thailand and Turkey.

#### A6.4.3 *Plasmodium malariae* resistance

##### Chloroquine

Resistance of *P. malariae* to chloroquine was observed recently in Indonesia.

## A6.5 Monitoring of antimalarial drug resistance

### A6.5.1 Monitoring methods

The rapid spread of antimalarial drug resistance over the last few decades has increased the need for monitoring, in order to ensure proper management of clinical cases, allow for early detection of changing patterns of resistance, and suggest where national malaria treatment policies should be revised. The monitoring procedures available include therapeutic efficacy testing (also known as *in vivo* testing). This involves the repeated assessment of clinical and parasitological outcomes of treatment – during a fixed period of follow-up – in order to detect any reappearance of symptoms and signs of clinical malaria and/or parasites in the blood, which would indicate reduced parasite sensitivity with the particular drug. Other methods include *in vitro* studies of parasite susceptibility to drugs in culture, and studies of point mutations or duplications in parasite resistance genes with molecular methods (polymerase chain reaction, PCR). Animal models are also used, although not routinely.

#### *In vivo*

#### **(a) Therapeutic efficacy testing and the WHO standard protocol for *P. falciparum***

From a programmatic point of view, data on therapeutic efficacy are most useful in deciding whether or not a drug is still appropriate as first-line treatment. Therapeutic efficacy studies are relatively simple to conduct, and the requirements in terms of training of staff and technical facilities are therefore limited. However, the results can be affected by misdiagnosis and incorrect drug administration. In order to interpret and allow for comparison of the results within and between regions, and to follow trends over time, studies need to be conducted according to similar procedures and standards. WHO therefore recommends the use of the WHO standard protocol, which provides guidance on how best to obtain the minimum necessary information about the therapeutic responses to an antimalarial so as to allow informed decision-making on its future use (49).

The protocol is designed for use in the assessment of antimalarial drugs or drug combinations used routinely for treatment of uncomplicated *P. falciparum* malaria (chloroquine, sulfadoxine-pyrimethamine, amodiaquine, artemisinin-based combination therapies and others). It comprises a simple, one-arm prospective evaluation of clinical and parasitological treatment responses in children aged 6–59 months, in whom the level of acquired immunity is relatively low and therefore has only a minor influence on the outcome of the

test. To ensure a reasonable specificity of the malaria diagnosis in areas of intense transmission, only individuals with a parasite density  $\geq 2000$  asexual parasites/ $\mu\text{l}$  of blood should be included in studies. In areas of low to moderate transmission, individuals with  $\geq 1000$  asexual parasites/ $\mu\text{l}$  can be included. Further methodological and operational considerations in relation to case definition, sample size calculations, ethical concerns and the criteria for inclusion and exclusion, some of which some relate only to specific drugs, are explained in detail in the protocol.

The recommended duration of follow-up is  $\geq 28$  days in areas of intense as well as low to moderate transmission. As a significant proportion of treatment failures do not appear until after day 14, shorter observation periods lead to a considerable overestimation of the efficacy of the tested drug. This is a particular problem at low levels of resistance and with low failure rates (50). As the objective of treatment is cure of the infection, and cure rates of more than 90% are required, the cure rate must be adequately characterized. For relatively effective, slowly eliminated antimalarials, half the recrudescences may occur after 28 days. For treatment with drugs such as amodiaquine, chloroquine and sulfadoxine-pyrimethamine, a 28-day follow-up is considered appropriate; follow-up periods of 42 days and 63 days are recommended for artemether-lumefantrine and mefloquine, respectively (51). These follow-up periods will capture most but often not all recrudescence infections – particularly at low levels of resistance. Studies even of  $> 28$  days of duration risk loss to follow-up and should be accompanied by molecular assessments (PCR genotyping) so as to distinguish recrudescence from reinfection. If surveillance programmes do not have access to molecular techniques, studies of 14 days of duration without PCR adjustments can still provide useful information on failing drugs (i.e. to justify their replacement) – but they cannot be used to justify inclusion or continued recommendation. In areas of low to moderate transmission, the use of molecular methods is recommended, but is not strictly essential if the likelihood of reinfection is relatively small. PCR genotyping involves comparison of polymorphic parasite genes, usually those encoding variable blocks within PfMSP2, and also sometimes PfMSP1 and PfGLURP, in whole blood samples taken during the acute and recurrent infections.

The WHO standard protocol classifies outcomes of efficacy studies into the following four categories: early treatment failure, late clinical failure, late parasitological failure, and adequate clinical and parasitological response. These classifications rely on the presence or absence of fever or other signs of clinical malaria and/or presence of parasitaemia during the course of follow-up (Table A6.1). The therapeutic response is classified as early treatment failure if the patient develops clinical or parasitological symptoms during the first 3 days of follow-up. The response is classified as late clinical failure

if symptoms develop during the follow-up period (from day 4 to day 28), without previously meeting the criteria for early treatment failure. It is a late parasitological failure if only parasitaemia reappear without any symptom, in the period from day 7 to day 28. Adequate clinical and parasitological response is defined as the absence of symptoms and of parasitaemia on day 28, without any of the criteria for the other three categories having been met previously.

**Table A6.1**

(49)

Treatment outcome	Symptoms and signs
Early treatment failure	<ul style="list-style-type: none"> <li>• Development of danger signs or severe malaria on days 1–3 in the presence of parasitaemia</li> <li>• Parasitaemia on day 2 higher than the day 0 count irrespective of axillary temperature</li> <li>• Parasitaemia on day 3 with axillary temperature <math>\geq 37.5</math> °C</li> <li>• Parasitaemia on day 3 that is <math>\geq 25\%</math> of count on day 0.</li> </ul>
<ul style="list-style-type: none"> <li>• Late clinical failure</li> </ul>	<ul style="list-style-type: none"> <li>• Development of danger signs or severe malaria after day 3 in the presence of parasitaemia, without previously meeting any of the criteria of early treatment failure</li> <li>• Presence of parasitaemia and axillary temperature <math>\geq 37.5</math> °C (or history of fever) on any day from day 4 to day 28, without previously meeting any of the criteria of early treatment failure.</li> </ul>
<ul style="list-style-type: none"> <li>• Late parasitological failure</li> </ul>	<ul style="list-style-type: none"> <li>• Presence of parasitaemia on any day from day 7 to day 28 and axillary temperature <math>&lt; 37.5</math> °C, without previously meeting any of the criteria of early treatment failure or late clinical failure.</li> </ul>
Adequate clinical and parasitological response	<ul style="list-style-type: none"> <li>• Absence of parasitaemia on day 28 irrespective of axillary temperature without previously meeting any of the criteria of early treatment failure, late clinical failure or late parasitological failure.</li> </ul>

For simplicity, the outcome of efficacy studies can be summarized as “clinical failure”, which is equal to the sum of early treatment failure and late clinical failure, and as “total failure”, which is equal to the sum of early treatment failure, late clinical failure and late parasitological failure. The rates of clinical failure and total failure are used to define cut-off points for drug policy change,

using the standard WHO protocol. It should be noted that the most recent classification of therapeutic responses described above differs from that used previously; late parasitological treatment responses are now also considered as an indicator of drug efficacy, as persistent parasitaemia is associated with increased risk of clinical malaria, anaemia and increased gametocyte carriage (52). The protocol provides guidance on how to calculate and present efficacy test results.

If feasible, any judgement of the therapeutic efficacy of a drug should be accompanied by measurements of blood drug concentrations, to ensure that therapeutic drug levels were reached; subtherapeutic levels confound the efficacy result. With modern techniques, antimalarial drug concentrations can often be analysed in small samples of blood dried on filter paper; samples can be sent to a central pharmacological laboratory for analysis.

#### **(b) *In vivo* assessment of resistance in *P. malariae***

Protocols similar to those used for *P. falciparum* can be used.

#### **(c) *In vivo* assessment of resistance in *P. vivax* and *P. ovale* infections**

Relapse and recrudescence cannot be distinguished reliably in these infections, as they will usually have the same genotype. Nevertheless the *in vivo* assessment of chloroquine susceptibility can be performed using the same format as for *P. falciparum*, with a follow-up period of 28 or preferably 35 days, and preferably accompanied by measurement of whole blood chloroquine and desethylchloroquine levels. Recurrent infections within this period presenting with whole blood chloroquine + desethylchloroquine concentrations exceeding 100 ng/ml can be considered as resistant whether they are a relapse, a recrudescence, or even a new infection, as this concentration should be suppressive (50, 53, 54).

#### ***In vitro***

To support evidence of a failing antimalarial, *in vitro* tests can be used to provide a more accurate measure of drug sensitivity under controlled experimental conditions. Parasites obtained from finger-prick blood are placed in microtitre wells, exposed to precisely known concentrations of a particular drug and examined for the inhibition of maturation into schizont parasite stages (55). This test overcomes some of the many confounding factors influencing the results of *in vivo* tests, such as subtherapeutic drug concentrations and the influence of host factors on parasite growth (e.g. factors related to acquired immunity), and therefore provide a more accurate picture of the “true” level of resistance to the drug. Multiple tests can be performed on parasite isolates,

using several drugs and drug combinations simultaneously. New experimental drugs can also be tested in this way. However, partly because *in vitro* tests do not include host factors, the correlation between results of *in vitro* and *in vivo* tests is not consistent and is not well understood. Furthermore, different parasite isolates may adapt differently to culture, which may affect the test result. For example, if a resistant strain adapts less well to culture and therefore dies off earlier, the outcome is an overestimation of its susceptibility. Prodrugs such as proguanil, which require conversion into active metabolites in the human host, cannot be tested, and *P. vivax*, *P. ovale* and *P. malariae* cannot be evaluated *in vitro* owing to constraints in culturing these species (although this has now largely been overcome for *P. vivax*). *In vitro* testing is more demanding in terms of technology and resources, and is not ideal for routine drug efficacy evaluation under field conditions. It should therefore primarily be used to provide additional information to support clinical efficacy data at selected resistance-monitoring sites.

In recent years, molecular tests have been developed for the detection of parasite gene mutations or amplifications associated with resistance to antimalarials as an additional means of assessing levels of drug resistance. These methods are based on PCR, using small amounts of parasite DNA material in finger-prick blood dried on filter paper.

Information on the prevalence of gene mutations may give an indication of the level of drug resistance in an area, and relatively well-defined molecular markers of resistance have been established for pyrimethamine (*Pfdhfr* and *Pvdhfr*), sulfadoxine (*Pfdhps*) and chloroquine (*Pfcr1*) (56, 57). Amplification of *Pfmdr* (for mefloquine resistance) is considerably more technically demanding, requiring validated real-time PCR. No markers are yet available for other antimalarials.

These methods have their disadvantages. The results are seldom available rapidly, and mutations and the measured therapeutic efficacy do not always correlate well, as many factors determine the therapeutic response in addition to parasite sensitivity to the antimalarial drug treatment. However, serial assessment of molecular markers can be a useful guide to the emergence of resistance, especially if used consistently over time in comparable study populations to detect trends. The methods may also provide useful guidance on choices of treatment during acute malaria epidemics, where time will not allow for clinical efficacy tests, but where it may be critical to avoid the use of a particular drug (58). The requirements for technical skills and laboratory facilities prevent the routine use of these methods in most drug efficacy

testing sites, although they are becoming increasingly used in laboratories in endemic areas – particularly those supporting clinical trials. Moreover, the results are subject to error and must be considered carefully. For example, a patient may have an infection with two different genotypes on day 0 but, only one genotype is detected with PCR. If one genotype is sensitive and the other is resistant, the resistant genotype may persist until day 14 despite antimalarial treatment, while the sensitive genotype will be cleared; if detected, the presence of the resistant genotype on day 14 may then incorrectly be interpreted as reinfection and not as recrudescence (59).

While monitoring of molecular markers may only be possible at central laboratories, it can support monitoring programmes that rely on *in vivo* testing, and also play an important part in early-warning systems to guide treatment policies coordinated at national and regional levels. With newly developed high-throughput methods (60, 61), more comprehensive population-based analyses will also be possible, which may allow for a better understanding and prediction of the future spread of resistance (40, 42).

In addition to *in vivo* efficacy studies involving human participants, drug sensitivity can be tested in animal models. Although such models do not play an important role in routine efficacy-monitoring programmes, they may be useful in the testing of newly developed drugs, not yet approved for use in humans, or to minimize the influence of host immunity on drug efficacy, while retaining the influence of some of the extrinsic factors observed in *in vivo* studies.

### A6.5.2 Reporting of treatment failures

Reports of cases of treatment failure and decreased drug sensitivity have often provided important first evidence for more widespread resistance in an area. Although such evidence is subject to bias, it can be collected without much effort at peripheral health centres. If standardized and registered, such reports can make a valuable contribution to national early-warning systems, facilitating cost-effective monitoring by national programmes.

### A6.5.3 Criteria for antimalarial drug policy change

The *WHO malaria treatment guidelines* recommend that antimalarial treatment policy should be changed at treatment failure rates considerably lower than those recommended previously. This major change reflects the availability of highly effective drugs, and the recognition both of the consequences of drug

resistance, in terms of morbidity and mortality, and of the importance of high cure rates in malaria control.

It is now recommended that a change of first-line treatment should be initiated if the total failure proportion exceeds 10%. However, it is acknowledged that a decision to change may be influenced by a number of other factors, including the prevalence and geographical distribution of reported treatment failures, health service provider and/or patient dissatisfaction with the treatment, political and economical contexts, and the availability of affordable alternatives to the commonly used treatment.

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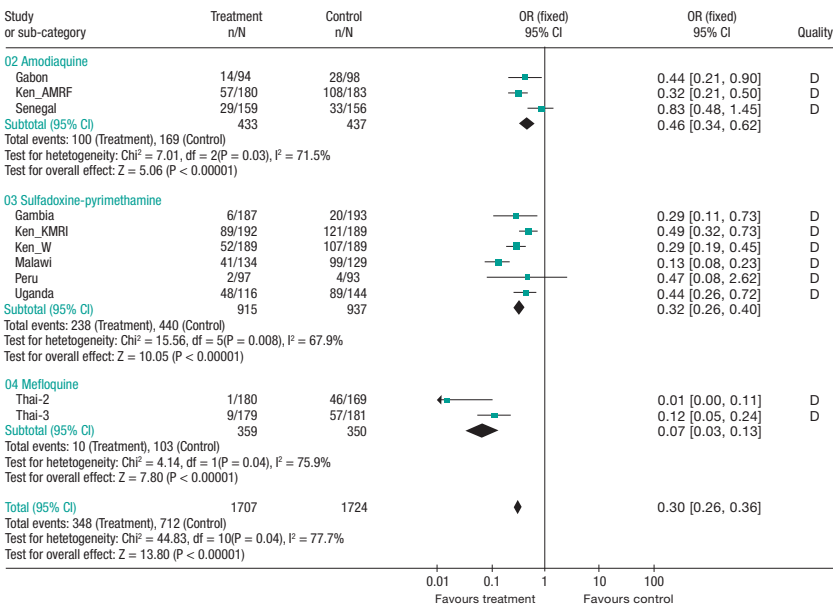
## ANNEX 7

UNCOMPLICATED *P. FALCIPARUM* MALARIA

## A7.1 How do artemisinin combination therapies compare with non-artemisinin monotherapies?

Systematic review and meta-analysis of individual patient data from 16 randomized trials (total of 5948 people) that studied the effects of the addition of artesunate to monotherapy for falciparum malaria (1) (search date, September 2002). The analysis compared odds ratios (OR) of parasitological failure at days 14 and 28 (artesunate combination compared to monotherapy) and calculated combined summary ORs across trials using standard methods. Results comprise parasite failure including re-infections by day 28 in 14 trials (Figure A7.1), and parasite failure excluding re-infections in 11 trials (Figure A7.2).

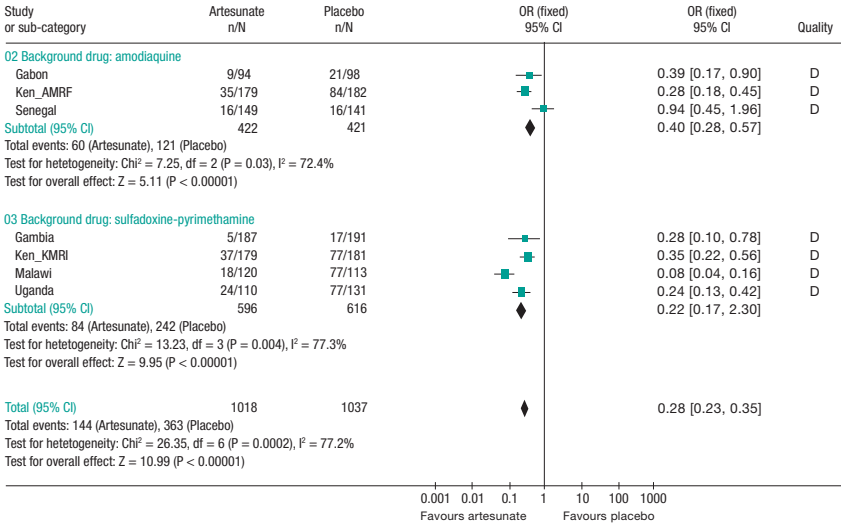
Review: Artesunate combination treatments for malaria  
 Comparison: 01 Artesunate combinations versus no artesunate  
 Outcome: 02 Parasitaemia by day 28 (all)



df, degrees of freedom; OR: Mantel-Haenszel odds ratio; CI: confidence interval

**Figure A7.1** Artemisinin derivatives administered in combination compared with monotherapy alone: total failures by day 28 (re-infections included); data from the International Artemisinin Study Group individual patient data meta-analysis (1)

Review: Artesunate combination treatments for malaria  
 Comparison: 01 Artesunate combinations versus no artesunate  
 Outcome: 02 Parasitological failure by day 28 (failure excludes re-infection)



df, degrees of freedom; OR: Mantel-Haenszel odds ratio; CI: confidence interval

**Figure A7.2** Artemisinin derivatives administered in combination compared with monotherapy alone: total failures by day 28 (reinfections excluded); data from the International Artemisinin Study Group individual patient data meta-analysis (16)

## A7.2 Are there any non-artemisinin combination therapies that provide an alternative to standard monotherapy?

### A7.2.1 Sulfadoxine-pyrimethamine + chloroquine compared with sulfadoxine-pyrimethamine

One systematic review (2) (search date 2001), which identified no randomized controlled trials (RCTs) meeting the criteria of at least a 28-day follow-up period for a clinical trial. No subsequent RCTs meeting the inclusion criteria but five with follow-up periods shorter than 28 days (3–7), which are described below.

The first subsequent RCT (160 children and adults, Colombia, 1999–2002), found a lower treatment failure rate at day 21 with the combination treatment than with sulfadoxine-pyrimethamine alone (11/64 (17%) with the combination, 19/79 (26%) with sulfadoxine-pyrimethamine alone, no statistical data reported) (3).

The second subsequent RCT (71 children, Uganda, 2001) found a lower treatment failure rate at day 14 with the combination treatment than with sulfadoxine-pyrimethamine alone (4/32 (13%) with the combination, 5/30 (17%) with sulfadoxine-pyrimethamine alone, no statistical data reported) (4).

The third subsequent RCT (52 children and adults, Lao People's Democratic Republic, 2001) found no significant difference in adequate clinical and parasitological responses at day 14 (20/24 with the combination, 23/28 with sulfadoxine-pyrimethamine alone; relative risk (RR): 1.01, 95% CI: 0.8–1.3) (5).

The fourth subsequent RCT (88 children and adults, Uganda, 2001) found no significant difference in adequate clinical response (27/27 with the combination, 29/29 with sulfadoxine-pyrimethamine alone) (6).

The fifth subsequent RCT (305 children and adults, Uganda, 2001–2002) found no significant difference in adequate clinical response (141/152 with the combination, 119/140 with sulfadoxine-pyrimethamine alone) (7).

Only one RCT reported on adverse events (7). It found that overall, the incidence of possible adverse events was higher in those receiving the combination therapy than in those receiving sulfadoxine-pyrimethamine monotherapy. This could be explained by the higher incidences of pruritus, nausea and vomiting in the combination group. When mild adverse events were excluded from the analysis, there was no significant difference between the groups. One severe adverse event was reported in the monotherapy group, namely an elevated alanine aminotransferase measurement on day 14 in a girl of 3 years of age. This event was not accompanied by symptoms and resolved within two weeks without medical intervention.

The systematic review (2) identified two RCTs comparing sulfadoxine-pyrimethamine + chloroquine to sulfadoxine-pyrimethamine alone (8, 9). However, neither trial met the WHO inclusion criteria.

The first RCT (85 children aged < 12 years, Papua New Guinea, 1980) compared combination treatment using sulfadoxine-pyrimethamine + an atypical single dose of chloroquine of 10 mg/kg daily to sulfadoxine-pyrimethamine alone (8).

The second RCT (405 children aged 1–10 years, The Gambia, 1995) had a high rate of loss to follow-up (30% in the sulfadoxine-pyrimethamine + chloroquine group, 26% in the monotherapy group) (9).

### A7.2.2 Sulfadoxine-pyrimethamine + amodiaquine compared with sulfadoxine-pyrimethamine

One systematic review (2) (search date 2001), which identified four RCTs, three in Africa (10–12) and one in China (13) (484 people). One subsequent RCT (14).

Three of the RCTs identified by the systematic review found slightly higher cure rates at day 28 with sulfadoxine-pyrimethamine + amodiaquine than with sulfadoxine-pyrimethamine alone, although the overall difference did not reach statistical significance (2). Similarly, two RCTs (one in Mozambique, and one in China, 1985–1986, 116 people) identified by the review found no significant difference in mean parasite clearance times between the two treatment groups (10, 13). However, the Chinese trial (69 people) found a significantly shorter mean fever clearance time with the combination treatment than with the monotherapy (13).

The subsequent RCT (191 children aged under 10 years, Cameroon, 2001) found a significantly higher adequate clinical response with a negative smear at day 28 with the combination treatment than with the monotherapy (14). Similarly, mean fever clearance time was significantly lower with the combination treatment than with the monotherapy.

The Chinese trial identified by the systematic review found no significant difference in the rate of adverse events between the two treatment groups (41.7% with the combination, 42.9% with the monotherapy); *p* value and absolute numbers not reported) (13). Sinus bradycardia and vomiting were the most frequent adverse events overall, and were also more frequent with the combination treatment than with sulfadoxine-pyrimethamine alone (no statistical data reported). However, abdominal pain, headache and dizziness were more common with the monotherapy (no statistical data reported). The Ugandan trial reported no serious adverse effects in either treatment group, whereas that conducted in Mozambique gave no information on adverse events (10, 11). The latter trial (400 people) measured haemoglobin and white blood cell count throughout the follow-up period and found no significant difference between the treatments (11).

The subsequent RCT found a significantly higher rate of fatigue, the most common adverse effect overall, with the combination treatment than with the monotherapy (59/62 (95%) with the combination, 47/62 (76%) with the monotherapy, *p* < 0.05). Similarly, there were higher rates of headache and

vomiting with the combination treatment than with the monotherapy (headache: 4/62 (6%) with the combination, 0/62 (0%) with the monotherapy,  $p < 0.05$ ; vomiting: 14/62 (23%) with the combination, 5/62 (8%) with the monotherapy,  $p < 0.05$ ). There was also an increased rate of pruritus with the combination treatment than with sulfadoxine-pyrimethamine alone, although the difference did not reach statistical significance (9/62 (15%) with the combination, 3/62 (5%) with the monotherapy,  $p$  value not reported). One person receiving sulfadoxine-pyrimethamine monotherapy presented with purulent vesicles in the thoracic region (no further information provided) (14).

### A7.2.3 Sulfadoxine-pyrimethamine + amodiaquine compared with amodiaquine

One systematic review (2) (search date 2001), which identified three RCTs, one each in China, Mozambique and Uganda) (10, 11, 13). Three subsequent RCTs (14–16).

Two RCTs (150 people in China, 1985–1986 (13) and Mozambique, 1986 (10) identified by the review found higher parasitological cure rates at day 28 with the combination treatment than with amodiaquine alone. Owing to the apparent heterogeneity between individual study results, the combined relative risk as assessed by several methods did not reach significance (see comment below). These trials found a slightly shorter mean parasite clearance time with the combination treatment than with the monotherapy, although the difference did not reach significance. Similarly, the Chinese trial (97 people) found a shorter mean fever clearance time with the combination treatment than with the monotherapy, although the difference did not reach statistical significance (13). The Ugandan trial (11) only reported parasite outcomes at day 7.

The first subsequent RCT (159 children aged 0.5–10.0 years, Nigeria, 2000–2001) found no significant difference in cure rates at day 28 or in mean fever clearance times between the two treatment groups. However, mean parasite clearance time was significantly shorter with the combination treatment than with amodiaquine alone (15).

The second subsequent RCT (127 children aged under 10 years, Cameroon, 2001) found a significantly higher adequate clinical response with negative smear at day 28 with the combination treatment than with amodiaquine alone. However, there was no significant difference in mean fever clearance time between the two groups (14).

The third subsequent RCT (235 children aged 6–59 months, Uganda, 2001) found a lower parasitological failure rate at day 28 with combination treatment (16).

The Chinese RCT identified by the review reported a slightly higher rate of adverse events with the combination treatment than with amodiaquine alone (41.7% with the combination, 36% with the monotherapy, *p* value not reported) (13). Sinus bradycardia and vomiting were the most frequent adverse events overall and were also more frequent with the combination treatment than with the monotherapy (no statistical data reported). The Ugandan RCT reported no serious adverse effects in either treatment group, whereas that conducted in Mozambique gave no information on adverse events (10, 11).

The first subsequent RCT found three children with sleep disturbance secondary to pruritus but there was no significant difference between the treatments (2/75 with the combination, 1/82 with the monotherapy, *p* value not reported). All other adverse reactions were reported as mild (15).

The second subsequent RCT found no significant difference in fatigue between treatments (59/62 (95%) with the combination treatment, 54/61 (89%) with amodiaquine alone, *p* value not reported). Cutaneous reactions (dermatitis in the hip area in one person and diffuse urticaria at day 5 in one person) were recorded in two people receiving the monotherapy (0/62 (0%) with the combination, 2/61 (3%) with the monotherapy, *p* value not reported) (14).

The third subsequent RCT reported both treatments to be well tolerated and found no serious adverse effects (16).

The systematic review showed significant differences in parasitological cure rates at day 28 between the two RCTs (10, 13). However, the reviewers found no significant difference between treatments when using a random effects model, or worst and best case scenarios assuming that people lost to follow-up were either all treatment failures or successes (2).

### A7.3. How do artemisinin combination therapies compare with non-artemisinin combinations?

#### A7.3.1 Artesunate + sulfadoxine-pyrimethamine

No systematic review. One RCT (276 children aged 6–59 months, Uganda, 2001) comparing artesunate (3 days) + sulfadoxine-pyrimethamine with amodiaquine + sulfadoxine-pyrimethamine (16). It found that parasitological failure at day 28 was significantly increased in the group receiving artesunate (3 days) + sulfadoxine-pyrimethamine compared to that in the group receiving amodiaquine + sulfadoxine-pyrimethamine (PCR-unadjusted treatment failure at day 28: 42/144 (29%) with artesunate (3 days) + sulfadoxine-pyrimethamine, 22/132 (17%) with amodiaquine + sulfadoxine-pyrimethamine; OR: 0.49; 95% CI: 0.27–0.87). However, it found no significant difference in treatment failure rates at day 28 between the two groups once new infections had been excluded (PCR-adjusted treatment failure at day 28: 17/132 (13%) with artesunate (3 days) + sulfadoxine-pyrimethamine, 29/134 (22%) with amodiaquine + sulfadoxine-pyrimethamine; OR: 0.59; 95% CI: 0.29–1.18,  $p = 0.14$ ).

The same RCT gave no information on adverse events (16).

### A7.4 Which is the best artemisinin combination therapy?

#### A7.4.1 Artemether-lumefantrine (6 doses) compared with artemether-lumefantrine (4 doses)

One RCT (17); the RCT (238 adults and children, Thailand, 1996–1997) found a significantly higher rate of cure at day 28 with the 6-dose regimen given over 3 days than with the 4-dose regimen also given over 3 days (PCR-unadjusted treatment cure rate for intention to treat population at day 28: 96/118 (81%); 95% CI: 73.1–87.9% with the 6-dose regimen, 85/120 (71%); 95% CI: 61.8–78.8% with the 4-dose regimen,  $p < 0.001$ ; PCR-adjusted treatment cure rate for evaluable population: 93/96 (97%); 95% CI: 91.1–99.4% with the 6-dose regimen, 85/102 (83%); 95% CI: 74.7–90.0% with the 4-dose regimen,  $p < 0.001$ ).

The RCT reported all adverse events to be mild or moderate in severity and possibly attributable to malaria (17). It found no adverse cardiovascular effects. It found four serious adverse events that the authors did not consider to be related to treatment. The trial found no changes in QRS duration and PR interval during treatment in 66 people who had regular electrocardiographic monitoring. Similarly, it found no differences in mean and median QTc values between treatment groups.

#### A7.4.2 Artemether-lumefantrine (6 doses) compared with artesunate (3 days) + mefloquine

One systematic review (search date 2004, two RCTs, 419 people, Thailand, 1997–1998 and 1998–1999) (18).

The review found a higher proportion of people with parasitaemia at day 28 with artemether-lumefantrine treatment than with artesunate-mefloquine although the pooled difference did not reach statistical significance (PCR-unadjusted parasitaemia rate 11/289 (4%) with artemether-lumefantrine, 0/100 (0%) with artesunate-mefloquine, RR: 4.20; 95% CI: 0.55–31.93,  $p = 0.2$ ; PCR-adjusted parasitaemia rate 9/289 (3%) with artemether-lumefantrine, 0/100 (0%) with artesunate-mefloquine, RR: 3.50; 95% CI: 0.45–27.03,  $p = 0.2$ ; see comment below). The first RCT (219 adults and children aged over 12 years, Thailand, 1998–1999) identified by the review found no significant difference in median parasite clearance time between the two treatment groups (29 h; 95% CI: 29–32 h in 164 people receiving artemether-lumefantrine, 31 h; 95% CI: 26–31 h in 55 people receiving artesunate-mefloquine,  $p$  value not reported) (19). Similarly, it found no significant difference in median fever clearance time (29 h; 95% CI: 23–37 h in 76 people receiving artemether-lumefantrine, 23 h; 95% CI: 15–30 h in 29 people receiving artesunate-mefloquine,  $p$  value not reported) or in median gametocyte clearance time between treatments (72 h; 95% CI: 34–163 h in 26 people receiving artemether-lumefantrine, 85 h; 95% CI: 46–160 h in 10 people receiving artesunate-mefloquine,  $p$  value not reported). The systematic review did not report results for any other outcomes from the second RCT.

The systematic review found fewer mild to moderate adverse events with artemether-lumefantrine than with artesunate-mefloquine, although the differences did not reach statistical significance (nausea 4/150 (3%) with

artemether-lumefantrine, 6/50 (12%) with artesunate-mefloquine; vomiting 4/150 (3%) compared to 5/50 (10%); sleep disorders: 2/150 (1%) compared to 8/50 (16%); dizziness: 8/150 (5%) compared to 18/50 (36%); *p* values not reported) (18). It found no significant difference in the proportion of people with severe adverse events between the two treatment groups (one person with each treatment).

#### A7.4.3 Artemether-lumefantrine (6 doses) compared with artesunate (3 days) + amodiaquine

No RCTs with a 28-day follow-up period but one (295 children under 5 years of age, Burundi, 2001–2002) with a 14-day follow-up period (20). The trial found no significant difference in the proportion of people with adequate clinical and parasitological response at day 14 between the treatments (140/141 (99.3%); 95% CI: 97.9–100.0% with artemether-lumefantrine, 142/149 (95.3%); 95% CI: 91.9–98.7% with artesunate-amodiaquine, *p* value not reported).

The RCT found no significant difference in adverse events between the treatment groups other than vomiting, which was significantly less frequent on days 1 and 2 with artemether-lumefantrine than with artesunate-amodiaquine (day 1, 5% with artemether-lumefantrine, 13% with artesunate-amodiaquine; day 2, 1% compared to 5%; *p* values not reported (20).

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## ANNEX 8

### MALARIA TREATMENT AND HIV/AIDS

Malaria and HIV/AIDS share determinants of vulnerability. Given the wide geographical overlap in occurrence and the resulting prevalence of co-infection, the interaction between the two diseases clearly has major public health implications (1). In sub-Saharan Africa, around 300 million cases of malaria occur annually and an estimated 25 million adults and children are living with HIV/AIDS. In 2003 in Africa, HIV/AIDS claimed the lives of an estimated 2.2 million people (2), and malaria 1 million, especially children (3). In South-East Asia, Latin America and the Caribbean there is also significant overlap of these two diseases.

#### A8.1 Epidemiological overlaps between malaria and HIV/AIDS

The impact of the interaction of malaria and HIV/AIDS is most apparent in areas with generalized HIV/AIDS epidemics and stable malaria. Sub-Saharan Africa carries a high burden of both diseases, thus co-infection is common in many areas. In the most severely affected countries (Central African Republic, Malawi, Mozambique, Zambia and Zimbabwe), more than 90% of the population are exposed to malaria and the prevalence of HIV infection in adults is over 10% (2). In contrast, southern Africa, which has a relatively low burden of malaria, is the worst affected subregion for HIV infection with prevalence as high as 30%. The frequent malaria epidemics in southern Africa may, however, increase the risk of dual infection.

In Latin America and the Caribbean, some overlap of malaria and HIV/AIDS occurs in the general population in Belize, Brazil, El Salvador, Guatemala, Guyana and Honduras. South-East Asian countries, such as Cambodia, Myanmar and Thailand, have a generalized HIV/AIDS epidemic but malaria distribution is heterogeneous in this region. Significant overlap is likely to occur in a number of Indian cities with urban malaria and increasing HIV transmission. Considering that an estimated one billion people in South-East Asia are exposed to unstable malaria, it is clear that even a small overlap of malaria and HIV/AIDS in these settings may have a large public health impact.

## A8.2 Evidence of interactions between malaria and HIV/AIDS

### A8.2.1 Impact of HIV/AIDS on malaria during pregnancy

There is substantial evidence of the effects of interactions between malaria and HIV/AIDS in pregnant women. HIV infection impairs the ability of pregnant women to control a *P. falciparum* infection (4, 5). They are more likely to develop clinical and placental malaria, more often have detectable malaria parasitaemia and have higher malaria parasite densities in peripheral blood (6, 7). Compared to women with either malaria or HIV infection, co-infected pregnant women are at increased risk of anaemia, preterm birth and intrauterine growth retardation (8, 9). As a result, children born to women with dual malaria and HIV infection are at high risk of low birth weight and death during infancy.

The presence of HIV/AIDS may result in a poorer response to treatment with antimalarials and to intermittent preventive treatment for malaria during pregnancy. Furthermore, there is a risk of adverse reactions if SP for the prevention of malaria in pregnant women and cotrimoxazole (a coformulation of trimethoprim and sulfamethoxazole) for prophylaxis against opportunistic infections are taken together, as both are sulfa-containing medicines.

### A8.2.2 Impact of HIV/AIDS on malaria in non-pregnant adults

Evidence on interactions between malaria and HIV/AIDS in non-pregnant adults is accumulating. In areas with stable malaria, HIV infection increases the risk of malaria infection and clinical malaria in adults, especially in those with advanced immunosuppression (10–12). In settings with unstable malaria, HIV-infected adults with AIDS are at increased risk of severe malaria and death (13, 14). Antimalarial treatment failure may be more common in HIV-infected adults with low CD4 cell counts than in those not infected with HIV (15, 16).

### A8.2.3 Effects of malaria on HIV infection

Acute malaria episodes cause a temporary increase in replication of HIV and hence in plasma viral load (17). However, so far there is no evidence that malaria has a substantial effect on the clinical progression of HIV infection, HIV transmission or response to antiretroviral treatment in areas where malaria and HIV overlap.

### A8.2.4 HIV/AIDS and malaria in children

Few studies have examined the interaction of malaria and HIV/AIDS in children (18, 19). However, HIV-infected children with advanced immunosuppression may have more episodes of clinical malaria and higher parasite densities than those whose immune status is less compromised. In areas of unstable malaria, HIV-infected children may be more likely to experience severe disease or coma (20).

### A8.2.5 Drug interactions

There are currently no documented clinical or pharmacological interactions between antimalarials and antiretrovirals. However, pharmacokinetic interactions between certain antimalarials and non-nucleoside reverse transcriptase inhibitors and protease inhibitors are theoretically possible and could lead to toxicity. This suggests that patients receiving protease inhibitors (and the non-nucleoside reverse transcriptase inhibitor delavirdine) should avoid halofantrine. Other antimalarials such as artemether-lumefantrine may also have the potential to interact with antiretrovirals.

Medicines used in the management of opportunistic infections in people living with HIV/AIDS may also interact with antimalarials (21). Interactions are possible between cotrimoxazole, which is used for prophylaxis of opportunistic infections, and SP, which is used for intermittent preventive treatment of malaria in pregnant women in some parts of Africa. It is recommended that SP should not be given if co-trimoxazole is being taken daily as this probably provides an equivalent antimalarial effect. While more research is required, emphasis should be placed on close monitoring and pharmacovigilance in the treatment of malaria and HIV/AIDS.

## A8.3 Implications for health systems and service delivery

In HIV-infected individuals, the use of a malaria case definition based on fever alone can result in a febrile illness that may be due to a wide range of ordinary, virulent and opportunistic infections being misdiagnosed and treated as malaria (22, 23). This may lead to inappropriate care of HIV-infected adults with severe febrile illnesses due to causes other than malaria. With the use of more costly antimalarials, it has become necessary to give greater emphasis to parasitological diagnosis (24), and this is particularly important in areas of high prevalence of HIV infection.

In areas affected by malaria and HIV/AIDS, integrated health services are crucial for the introduction of new drugs and diagnostic materials, and offer opportunities for joint planning, training and service delivery.

## A8.4 Key recommendations

- HIV-infected pregnant women in areas with stable malaria should – depending on the stage of HIV infection – receive either intermittent preventive treatment for malaria with at least three doses of sulfadoxine-pyrimethamine or daily cotrimoxazole prophylaxis for HIV/AIDS opportunistic infections. Malarial illness in HIV-infected pregnant women who are receiving cotrimoxazole prophylaxis should be managed with non-sulfa antimalarials.
- In areas with stable malaria and a high prevalence of HIV infection, use of a fever-based malaria case definition may result in febrile illnesses caused by opportunistic infections being misdiagnosed as malaria, leading to overtreatment of malaria. Confirmatory parasitological testing for malaria should be applied with high priority in patients at risk of HIV/AIDS (in particular in older children and adults). In addition, health providers should offer HIV testing and counselling.
- In countries with generalized HIV/AIDS epidemics, routine monitoring of antimalarial drug efficacy or effectiveness should include assessment of the effect of HIV on antimalarial treatment outcomes.
- Further research should be undertaken to evaluate possible interactions between antimalarials and antiretrovirals, and pharmacovigilance should be introduced to monitor adverse drug reactions for both the new antimalarials and antiretrovirals.

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## ANNEX 9

### TREATMENT OF SEVERE *P. FALCIPARUM* MALARIA

#### A9.1 Is a loading dose of quinine (20 mg/kg) superior to no loading dose?

One systematic review, and one subsequent randomized controlled trial (RCT) in children found no significant difference in mortality between quinine regimens with a high initial quinine dose and those with no loading dose. However, parasite and fever clearance times were reduced in the former.

One systematic review (search date 2002, three RCTs, 92 people) (1). One subsequent RCT (2).

The systematic review found no significant difference in mortality between a group receiving a high initial dose of quinine (20 mg salt/kg or 16 mg base/kg given by the intramuscular (i.m.) route or by i.v. infusion) followed by a standard dose of quinine, and one receiving the standard dose but no loading dose (two RCTs; 2/35 (5.7%) died in the group receiving a high initial dose, 5/37 (13.5%) with no loading dose; RR: 0.43; 95% CI: 0.09–2.15) (1). One of the RCTs (39 children) found no significant difference between the two groups in mean time to recover consciousness (14 h with a high initial dose, 13 h with no loading dose, weighted mean difference (WMD) 1.0 h; 95% CI: –8.8 h to +10.8 h) (3). Parasite clearance and fever clearance times were shorter for the high initial dose group than for the group with no loading dose (parasite clearance time: two RCTs, 67 people, WMD 7.4 h; 95% CI: –13.2 h to –1.6 h; fever clearance time, two RCTs, 68 people, WMD –11.1 h; 95% CI: –20.0 h to –2.2 h). The subsequent RCT (72 children aged 8 months to 15 years in Togo, 1999–2000) found no significant difference between the group receiving a high initial dose of i.v. quinine (20 mg salt/kg over 4 h, then 10 mg salt/kg every 12 h) and that receiving no loading dose (15 mg salt/kg every 12 h) in mortality (2/35 (6%) with a high initial dose, 2/37 (5%) with no loading dose, RR: 1.06; 95% CI: 0.16–7.1) (2). It also found no significant difference between the two groups for recovery of consciousness (35.5 h with a high initial dose, 28.6 h with no loading dose, WMD: +6.9 h; 95% CI: –0.6 h to +14.4 h) or time to 100% parasite clearance (48 h compared to 60 h).

The systematic review found no significant difference between the groups receiving a high initial dose of quinine and no loading dose in rate of hypoglycaemia (two RCTs; 4/35 (11%) hypoglycaemia with a high initial dose, 3/37 (8%) with no loading dose, RR: 1.39; 95% CI: 0.32–6.00) (1). In one RCT (33 people) included in the review, transient partial hearing loss was significantly increased in the group receiving a high initial dose (10/17 (59%) compared to 3/16 (19%), RR: 3.14; 95% CI: 1.05–9.38) (4). In another (39 children), there was no significant difference between the two groups in neurological sequelae (1/18 (6%) with a high initial dose, 2/21 (10%) with no loading dose, RR: 0.58; 95% CI: 0.06–5.91) (3).

## A9.2 Is intramuscular quinine as effective as intravenous quinine?

One RCT in children found no significant difference between i.m. and i.v. quinine in recovery times or death. However, the study may have lacked the power to detect clinically important differences between treatments.

No systematic review. One RCT (59 children aged <12 years, Kenya, 1989-1990), which compared i.m. quinine (20 mg salt/kg loading immediately followed by 10 mg salt/kg every 12 h) with standard-dose quinine given by i.v. infusion (10 mg salt/kg every 12 h) in severe falciparum malaria (3). The trial found no significant difference in mortality, mean parasite clearance time or recovery time to drinking or walking, but may have lacked the power to detect a clinically important difference (mortality: 3/20 (15%) deaths with i.m. quinine, 1/18 (5.6%) with i.v. quinine, RR: 2.7; 95% CI: 0.3–23.7; mean parasite clearance time: 57 h compared to 58 h, WMD: –1.0 h; 95% CI: –12.2 h to +10.2 h; mean recovery times to drinking 47 h compared to 32 h, WMD: +15 h; 95% CI: –5.6 h to +35.6 h; mean recovery times to walking: 98 h compared to 96 h, WMD: +2.0 h; 95% CI: –24.5 h to +28.5 h).

Neurological sequelae were reported in two children in the i.m. group, and one child in the i.v. group had transient neurological sequelae that were not specified (2/20 (10%) compared to 1/18 (5.6%), RR: 1.8; 95% CI: 0.2–18.2) (3).

### A9.3 Is intrarectal quinine as effective as intravenous or intramuscular quinine?

One systematic review of 8 trials detected no difference in effect on parasites, clinical illness between the rectal group and either i.m or i.v. groups. Some studies however excluded patients with severe malaria.

One systematic review (search date 2005, eight RCTs, 1247 people) (5). Five trials compared intrarectal quinine with i.v quinine infusion, while 6 compared with intramuscular quinine. The systematic review found no significant difference between intrarectal with i.v. or i.m. routes for death, parasite clearance by 48 hours and 7 days, parasite clearance time, fever clearance time, coma recovery time, duration of hospitalization, and time to drinking. However trials reporting these outcomes were small, which resulted in large confidence intervals for all the outcomes.

No reported rectal irritation. Mucoid stools, however, were reported in 4 patients receiving rectal quinine. No statistically significant difference between painful swelling at the site of application or pain at the site of application after administration (5).

### A9.4 Is intravenous artesunate superior to intravenous quinine?

One large multi-centre trial from South-East Asia enrolling 1461 patients (including 202 children less than 15 years old) found a significant advantage of artesunate over quinine in mortality (15% vs 22%). There was an absolute reduction in mortality of 34.7% (95% CI: 18.5–47.6%;  $P = 0.002$ ) in the artesunate group. Quinine was associated with hypoglycaemia (RR: 3.2,  $P = 0.009$ ).

No systematic reviews: two RCTs. The first (113 adults with severe malaria, Thailand) comparing i.v. artesunate (2.4 mg/kg initially, 1.2 mg/kg 12 h later, then 1.2 mg/kg daily) to i.v. quinine (20 mg/kg initially, then 10 mg/kg every 8 h) (6). It found no significant difference between the treatments in mortality

after 300 h (7/59 (12%) artesunate, 12/54 (22%) quinine, RR: 0.53; 95% CI: 0.23–1.26). It found that artesunate significantly improved parasite clearance time, but that there was no significant difference in fever clearance time or coma recovery time (parasite clearance time: 63 h with artesunate, 76 h with quinine,  $p = 0.019$ ; fever clearance time: 41 h compared to 65 h,  $p = 0.2$ ; coma recovery time: 17 h compared to 18 h,  $p = 0.6$ ).

The second RCT is a large multi-centre trial (SEAQUAMAT study group: Bangladesh, India, Indonesia and Myanmar) with 1431 patients enrolled (7). It found that mortality of 15% (107/730 in the artesunate group was significantly lower than the 22% (164/731) in the quinine group. An absolute reduction of 34.7% (95% CI: 18.5–47.6%;  $P = 0.0002$ ) in the artesunate group. There are, however, still insufficient data for children, particularly from high transmission settings.

The first RCT found that artesunate significantly reduced hypoglycaemia compared with quinine (6/59 (10%) compared to 15/54 (28%), RR: 0.37; 95% CI: 0.15–0.88) (6). One person treated with artesunate developed an urticarial rash. A similar finding was obtained in the second RCT where treatment with artesunate was well tolerated, whereas quinine was associated with hypoglycaemia (RR: 3.2; 95% CI: 1.3–7.8;  $P = 0.009$ ) (7).

## A9.5 Is intramuscular artemether as effective as intravenous quinine?

Two systematic reviews and three subsequent RCTs found no significant difference in death rates between the groups receiving artemether and quinine for severe malaria.

Two systematic reviews (8, 9) and three subsequent RCTs (10–12). The first review (search date not reported, seven RCTs, 1919 adults and children) analysed individual participant data (8). It found no significant difference in mortality between i.m. artemether and quinine given by i.v. infusion or i.m. injection (the latter in one RCT only) in severe falciparum malaria (mortality 136/961 (14%) with artemether, 164/958 (17%) with quinine; OR: 0.80; 95% CI: 0.62–1.02). Parasite clearance was faster with artemether than with quinine (OR: 0.62; 95% CI: 0.56–0.69). The review found no significant difference in

the speed of coma recovery, fever clearance time or neurological sequelae between artemether and quinine (coma recovery time, OR: 1.09; 95% CI: 0.97–1.22; fever clearance time, OR: 1.01; 95% CI: 0.90–1.15; neurological sequelae, OR: 0.82; 95% CI: 0.59–1.15).

The second review (search date 1999, 11 RCTs, 2142 people) found a small significant reduction in mortality for i.m. artemether compared with i.v. quinine (OR: 0.72; 95% CI: 0.57–0.91) (9). However, more rigorous analysis excluding three poorer quality trials found no significant difference in mortality (OR: 0.79; 95% CI: 0.59–1.05). The review found no significant difference in neurological sequelae at recovery between the artemether and quinine groups (OR: 0.8; 95% CI: 0.52–1.25).

The first subsequent RCT (105 people aged 15–40 years with cerebral malaria in Bangladesh) compared i.m. artemether (160 mg initially, then 80 mg/kg once daily) with i.v. quinine (loading dose 20 mg/kg, then 10 mg/kg every 8 h) (10). It found no significant difference in death rates between the artemether and quinine groups (9/51 (18%) compared to 10/54 (19%), OR: 0.94; 95% CI: 0.35–2.55). Mean fever clearance time and coma recovery time were significantly longer for artemether than for quinine (fever clearance time: 58 h compared to 47 h, WMD: 11.0 h; 95% CI: 1.6–20.4 h; coma recovery time: 74 h compared to 53 h, WMD: 20.8 h; 95% CI: 3.6–38.0 h). There was no significant difference in mean parasite clearance time between artemether and quinine (52 h compared to 61 h, WMD: 8.6 h; 95% CI: 22.5 h to +5.3 h).

The second subsequent RCT (41 children with severe malaria in Sudan, 40 analysed) compared i.m. artemether (3.2 mg/kg loading dose, then 1.6 mg/kg once a day) with i.v. quinine (loading dose 20 mg/kg, then 10 mg/kg every 8 h) (11). It found that artemether significantly increased fever clearance time but found no significant difference between artemether and quinine in time to parasite clearance (mean fever clearance time: 30.5 h with artemether, 18 h with quinine,  $p = 0.02$ ; mean parasite clearance time: 16 h compared to 22.4 h,  $p > 0.05$ ). There were no deaths in the artemether group, one child died with quinine (0/20 (0%) compared to 1/21 (5%),  $p$  value not reported).

The third subsequent RCT (77 comatose children aged 3 months–15 years with cerebral malaria) compared i.m. artemether (1.6 mg/kg every 12 h) with i.v. quinine (10 mg/kg every 8 h) (12). It found no significant difference in death rates between the artemether and quinine groups (3/38 (8%) compared to 2/39 (5%),  $p$  value not reported). There was no significant difference between the two groups in mean fever clearance time, coma recovery time and parasite clearance time (fever clearance time 31 h compared to 36 h; coma recovery time: 21 h compared to 26 h; parasite clearance time 36 h compared to 41 h;  $p$  values not reported for any comparison).

The two systematic reviews (8, 9) and one of the subsequent RCTs (3) found no significant difference in neurological sequelae between the artemether and quinine groups (systematic reviews, see the Benefits section; subsequent trial 3/51 (6%) with artemether, 1/54 (2%) with quinine, RR: 3.18; 95% CI: 0.34–29.56). However, in the first review, rates for the combined outcome of death or neurological sequelae were lower for artemether than for quinine (OR: 0.77; 95% CI: 0.62–0.96,  $p = 0.02$ ) (8).

The second subsequent RCT found that one child treated with quinine developed hypoglycaemia (0/20 (0%) with artemether, 1/21 (5%) with quinine, (11). It reported no neurological problems in either treatment group after 28 days of follow-up.

The third subsequent RCT found no significant difference in transient neurological sequelae between the artemether and quinine groups (2/38 (5%) compared to 1/39 (3%), (12).

The third subsequent randomized controlled trial did not use loading doses of either artemether or quinine at the beginning of treatment (12). There was a fourth subsequent RCT (52 people) (13). However, it was not clear whether participants had severe malaria, and outcomes were poorly reported.

## A9.6 Is intramuscular artemotil as effective as intravenous quinine?

Cochrane Review (search date August 2004). Two small trials ( $n = 194$ ) met the inclusion criteria (14). Both trials compared i.m. artemotil with quinine given by i.v. infusion in children with cerebral malaria and reported on similar outcomes. There was no statistically significant difference in the number of deaths (RR: 0.75; 95% CI: 0.43–1.30,  $n = 194$ , 2 trials), neurological complications (RR: 1.18; 95% CI: 0.31–4.46,  $n = 58$ , 1 trial), or other outcomes including time to regain consciousness, parasite clearance time and fever clearance time. The meta-analyses lack the statistical power to detect important differences.

## A9.7 Is rectal artemisinin as effective as intravenous quinine?

One systematic review of small RCTs found no significant difference in mortality between rectal artemisinin and quinine given by i.v. infusion.

One systematic review (search date 1999, three RCTs) comparing rectal artemisinin with i.v. quinine in severe malaria (9). Two RCTs were conducted in 1996–1997. Meta-analysis showed lower mortality with artemisinin and quicker coma recovery time, but the difference was not significant (mortality, three RCTs, 9/87 (10%) with artemisinin, 16/98 (16%) with quinine, RR: 0.73; 95% CI: 0.35–1.50; coma recovery, two RCTs, 59 people, WMD: –9.0 h; 95% CI: –19.7 h to +1.7 h). Fever clearance times were not significantly different (no figures provided).

One RCT in children found that artemisinin significantly reduced the risk of hypoglycaemia compared with quinine (3/30 (10%) with artemisinin, 19/30 (63%) with quinine, RR: 0.16; 95% CI: 0.05–0.48) (15).

## A9.8 Pre-referral treatment with rectal artesunate: should rectal artesunate be used in preference to quinine?

There are no data from trials with sufficient statistical power to assess differences in mortality following treatment with rectal artesunate and quinine in people with moderate or severe malaria. The objective of the trials that have been conducted was to establish the safety and efficacy of rectal artesunate as pre-referral treatment where there is no access to parenteral treatment. Comparisons between rectal artesunate and i.v. artesunate or i.v., i.m. quinine have been carried out to assess parasitological and clinical response in the 12 or 24 hours immediately after treatment (16, 17).

Two randomized, open-label Phase II and three randomised open label Phase III studies have been conducted in people with moderately severe malaria, i.e. patients who could not take drugs by mouth but did not have features of severe

malaria and its complications (16, 17). Patients in the artesunate group in the Phase III studies were rescued if their parasitaemia did not decline to below 60% of baseline parasitaemia or if they deteriorated clinically and developed features of severe malaria, convulsions or coma within 24 hours of treatment.

Artesunate had a superior effect in all efficacy measures immediately after treatment. In children treated with artesunate, 80/87 (92%) had a parasite density lower than 60% of baseline, compared to 3/22 (14%) of those who received quinine (RR: 0.09; 95% CI: 0.04–0.19,  $p < 0.0001$ ). In adult, parasitaemia at 12 hours was lower than 60% of baseline in 26/27 (96%) in the artesunate group, compared to 3/8 (38%) in the quinine group. (RR: 0.06; 95% CI: 0.01–0.44,  $p < 0.001$ ). The differences were more significant at 24 h. Artesunate and/or dihydroartemisinin were detected in plasma within 12 h in all adults and in 84/87 of the children.

A single administration of artesunate suppositories at a dose of 10 mg/kg was well tolerated in both children and adults. There was no significant difference in frequency of adverse events (defined as any new symptom, worsening of any existing symptom, sign or abnormal laboratory value) between treatment groups. Other than local reactions at the site of the i.m. quinine injection in three adult patients, the few adverse events that occurred could have been attributable to falciparum malaria or to pre-existing disease.

## A9.9 Should dexamethasone be given routinely?

One systematic review found no significant difference in mortality between dexamethasone and placebo, but gastrointestinal bleeding and seizures were more common with dexamethasone.

One systematic review (search date 1999, two RCTs, 143 people with severe cerebral malaria treated with quinine), which compared dexamethasone with placebo over 48 h (18). One RCT was conducted in Indonesia, the other in Thailand. The review found no significant difference in mortality (14/71 (20%) with dexamethasone, 16/72 (25%) with placebo, RR: 0.89; 95% CI: 0.47–1.68). One RCT found a longer mean time between start of treatment and coma resolution with dexamethasone (76.0 h compared to 57.0 h,  $p < 0.02$ ) (18), but the other found no significant difference (83.4 h compared to 80.0 h, WMD: +3.4 h; 95% CI: –31.3 h to +38.1 h) (20).

The review found that dexamethasone significantly increased gastrointestinal bleeding and seizures compared with placebo (gastrointestinal bleeding 7/71 (10%) with dexamethasone, 0/72 (0%) with placebo, RR: 8.17; 95% CI: 1.05–63.6; seizures 1/71 (15.5%) compared to 3/72 (4%), RR: 3.32; 95% CI: 1.05–10.47) (18).

No effect of the steroid on mortality was shown, but the trials were small. Its effect on disability was not reported.

### A9.10 Is an initial blood transfusion effective for treating malarial anaemia?

One systematic review found insufficient data to be sure whether routine administration of blood to clinically stable children (no respiratory distress or cardiac failure) with severe anaemia in endemic malarious areas reduces death, or results in higher haematocrit measured at one month. The review found no significant difference between transfusion and no transfusion for the combined outcome of death or severe adverse events. Transmission of hepatitis B or HIV was not reported. No RCTs examining the effects of transfusion in adults with malaria.

One systematic review (search date 1999, RCTs, 230 children with malarial anaemia; packed cell volume range 12–17%) (21). The first RCT (116 children, United Republic of Tanzania) compared initial blood transfusion with conservative treatment, and the second (114 children, The Gambia) compared blood transfusion with iron supplements. Both trials excluded children who were clinically unstable with respiratory distress or signs of cardiac failure. Meta-analysis found fewer deaths in the transfused children, but the difference was not significant (1/118 (1%) with transfusion, 3/112 (3%) with control, RR: 0.41; 95% CI: 0.06–2.70). No RCTs examining the effects of transfusion in adults with malaria.

The systematic review found that coma and convulsions occurred more often after transfusion (8/118 (6.8%) with transfusion, 0/112 (0%) without, RR: 8.6; 95% CI: 1.1 to 66.0) with seven of the eight adverse events occurring in one of

the RCTs. Meta-analysis combining deaths and severe adverse events found no significant difference between children who received transfusions and those who did not (8/118 (7%) with transfusion, 3/112 (3%) without transfusion, RR: 2.5; 95% CI: 0.7–9.3). Transmission of hepatitis B or HIV was not reported (21).

Studies were small and loss to follow-up was greater than 10%; both of these factors are potential sources of bias. In the first RCT, one child in the transfusion group and one child in the conservative treatment group required an additional transfusion after clinical assessment. In the second RCT, 10 children allocated to receive iron supplements later required transfusion when packed cell volume fell below 12% or they showed signs of respiratory distress.

### A9.11 Should phenobarbital be given to patients?

Cochrane Review, search date 2004 (22). Three RCTs with a total of 573 participants met the inclusion criteria. All three compared phenobarbital with placebo or no treatment. In the two trials with adequate allocation concealment, death was more common in the anticonvulsant group (RR: 2.0; 95% CI: 1.20–3.33, fixed-effect model). In all three trials, phenobarbital was associated with fewer convulsions than placebo or no treatment (RR: 0.30; 95% CI: 0.19–0.45, fixed-effect model).

### A9.12 Hyperparasitaemia in non-immune or semi-immune populations

Since the classic paper by Field and Niven in 1937 (23), patients with high parasite counts have been known to be at increased risk of dying. Working in Peninsular Malaysia they showed that *P. falciparum* parasite counts of > 100 000/μl of blood (2%) were associated with an increased risk of mortality, while patients with counts of > 500 000/μl had a more than 50% chance of dying. Following this observation, hyperparasitaemic patients have been considered to be at high risk. They are often classified as having severe malaria and accordingly treated with parenteral antimalarial drugs wherever possible.

Many hyperparasitaemic patients have evidence of vital organ dysfunction but there is a subgroup of individuals in whom no other manifestations of severe disease occur. These patients have symptoms and signs compatible with a diagnosis of uncomplicated malaria in association with a high parasite count.

They usually have a predominance of young ring forms in the peripheral blood smear, which suggests that the sequestered biomass is relatively small compared with the circulating parasite numbers. On the Thai-Burmese border, the mortality of falciparum malaria in patients with counts of parasitized red blood cells  $<4\%$  was approximately  $0.1\%$ , whereas with counts  $>4\%$  but without vital organ dysfunction, the mortality was  $3\%$  (24). In another study in eastern Thailand, 48 hyperparasitaemic patients were inadvertently included in a cohort of 224 patients (mainly adult men) being followed up after receiving mefloquine treatment for uncomplicated disease in order to identify predictors of treatment failure. Young age, low admission haemoglobin, a history of diarrhoea at the start of treatment or soon after, three or more mefloquine treatments within the preceding year and hyperparasitaemia on an admission blood film were all predictors of subsequent treatment failure (25). The study took place at a time when mefloquine resistance was becoming well established. The authors concluded that, in areas of multidrug resistance, the initial parasite load becomes a critical determinant of treatment failure because strains have intermediate susceptibility to drugs. Patients with little or no immunity to malaria (i.e. children) are at increased risk of treatment failure. In a review of the predictors of treatment failure in a large series of patients studied on the western border of Thailand, the relative risk of treatment failure was increased in patients with parasite counts  $>10\ 000/\mu\text{l}$  (RR: 1.36; 95% CI: 1.12–1.66) (25). In Uganda, it was found that pretreatment parasitaemia  $>100\ 000/\mu\text{l}$  was associated with treatment failure in adults (OR for failure: 2.26; 95% CI: 1.30–3.92), but the authors stated that this was due to a strong association with body temperature in a multivariate analysis (26).

There is no uniformly agreed definition of hyperparasitaemia. Some publications use a parasite count  $>100\ 000/\mu\text{l}$  of blood. Others use a proportion of parasitized red blood cells  $>4\%$ ,  $>5\%$  or  $>10\%$ . In areas of high malaria transmission, mortality rates in patients with  $4\%$  or  $5\%$  parasitaemia are considerably lower than in areas of low transmission because of the influence of host immunity.

### *Is parenteral treatment better?*

There are very few studies specifically looking at treatment failure as an outcome in hyperparasitaemic patients. A study working with hyperparasitaemic ( $>4\%$ ) patients in a low-transmission setting, compared oral (artesunate for 3 days + mefloquine on day 2) with i.v. therapy (loading-dose quinine regimen for 24 h followed by mefloquine). Patients in the oral therapy group had

significantly faster parasite and fever clearance and shorter hospital stays than those in the intravenous therapy group. No patients progressed to severe disease. However, 28-day cure rates for the oral therapy group were poorer than in non-hyperparasitaemic controls receiving the same treatment (70% compared to 96%). It was concluded that patients with hyperparasitaemia in areas of multidrug resistance need a longer course of treatment to deal with the larger parasite biomass (27).

In one study from a high-transmission setting, 84 hyperparasitaemic (>5%) children aged 1–10 years were assigned at random to receive either 5 days of i.m. artemether or a single oral dose of mefloquine. Cure rates assessed at day 14 were similar in the two groups (98% at day 14 for artemether, 100% at day 28 for mefloquine), although parasite clearance was faster in the artemether group. Four patients in the mefloquine group were excluded because of vomiting, but no patients progressed to severe disease. The authors concluded that oral therapy may be given safely to hyperparasitaemic patients in Africa provided that they can tolerate it orally and the parasites are fully sensitive to the drug (28).

Another study from an area of stable transmission showed rapid parasite clearance in all 32 patients with parasitaemias of 5–35% (mean 10.8%) following oral amodiaquine therapy. Follow-up was to day 14 only and there was no comparative treatment arm. The authors concluded that hyperparasitaemic patients can safely be treated with oral therapy if they have no features of severe disease and can be closely monitored in the first 3 days (29). The same conclusion was reached from another cohort study from an area of stable malaria transmission (30).

### *Is duration of treatment important?*

A study of 100 hyperparasitaemic (>4%) adults and children on the Thai-Burmese border compared treatment with oral artesunate given alone for 5 days with artesunate given for 5 days + mefloquine on day 2. Recrudescence (i.e. treatment failure) rates were 36% and 6% respectively. Two further hyperparasitaemic treatment groups were then investigated: 34 patients with recrudescence following treatment with artesunate + mefloquine who then received artesunate for 7 days; and 132 patients from a malaria vaccine study with primary infections who received artesunate for 7 days + mefloquine on day 2. In these two groups, which were non-randomized and not strictly comparable, recrudescence rates were 26% and 7%, respectively. The authors concluded that duration of treatment is important, particularly when using artemisinin derivatives with short half-lives; that the addition of mefloquine improved cure rates; and that it is important that the parasite counts are

reduced massively by artesunate before using mefloquine so that selection pressure for resistance to mefloquine can be minimized (31).

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There have been no treatment studies looking at these patients specifically. They are often treated as having severe malaria even if they can take oral treatment. In low-transmission settings this group of patients was excluded from hyperparasitaemia studies because of the risks of clinical deterioration. Current practice for patients with parasitaemia >20% in these settings is to give parenteral artesunate (or parenteral quinine if artesunate is not available), but this approach is not based on evidence (E. Ashley, personal communication). In Nigeria, where there are higher transmission rates, an upper limit to hyperparasitaemia was not used but the patients with extreme hyperparasitaemia were not analysed as a separate group, so no data specific to this group are available.

Hyperparasitaemia carries an increased risk of mortality in falciparum malaria. The activity of artemisinin derivatives against circulating parasites makes them particularly effective in hyperparasitaemic patients who have no other signs of severity. Treatment failure rates are higher in hyperparasitaemic patients. This is also a potential source of new drug resistance.

Hyperparasitaemic patients with no signs of severe disease may be treated with oral artemisinin derivatives provided that the following apply:

- Patients must be monitored closely for the first 48 h after initiation of treatment.
- They must tolerate the drug(s) well, i.e. without diarrhoea or vomiting.
- For regimens containing mefloquine, the mefloquine should be given on day 2 when it is better tolerated, with a lower incidence of early vomiting, rather than on day 0.
- If possible, additional oral artemisinin derivative should be given so that the total treatment course is 5–7 days. This has been studied only for artesunate + mefloquine (artesunate 4 mg/kg immediately then 2 mg/kg/day for a further 4–6 days + mefloquine 25 mg/kg given as a split dose after the second day). Alternatively, the first dose of artemisinin derivative can be given parenterally or rectally to ensure adequate absorption.

Non-immune patients with parasitaemia >20% should continue to receive parenteral therapy wherever possible, as there is no evidence for or against using oral treatment in this group and the risks are high.

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## ANNEX 10

### TREATMENT OF *P. VIVAX*, *P. OVALE* AND *P. MALARIAE* INFECTIONS

#### A10.1 Introduction

*P. vivax*, the second major human malaria species, constitutes about 41% of malaria cases worldwide (1, 2) and is the dominant species of malaria in many areas outside Africa. It is prevalent in the Middle East, Asia, the Western Pacific and Central and South America. It is rarer in Africa and almost absent from West Africa (2). In most areas where *P. vivax* is prevalent, malaria transmission rates are low and, therefore, the affected populations achieve little immunity to this parasite. Consequently, people of all ages, adults and children alike are at risk of acquiring *P. vivax* infections (2). Where both *P. falciparum* and *P. vivax* prevail, the incidence rates of *P. vivax* tend to peak in people of a younger age than those of *P. falciparum* (3). The other two human malaria parasite species, *P. malariae* and *P. ovale*, are generally much less prevalent worldwide.

Among the four species of *Plasmodium* that affect humans, only *P. vivax* and *P. ovale* have the ability to form hypnozoites, parasite stages in the liver that can result in relapse infections weeks to months after the primary infection. *P. vivax* preferentially invades reticulocytes and this may lead to anaemia. Repeated infections lead to a chronic anaemia that can affect personal well-being, thereby impairing human and economic development in affected populations. The residual malaria burden of *P. vivax* is likely to be underestimated, and is increasing in some regions of the world (2). Appropriate case management of *P. vivax* malaria will help to minimize the global malaria burden.

Although *P. vivax* is known to be benign malaria, it causes a severe and debilitating febrile illness. Vivax malaria can also occasionally result in severe disease with life-threatening end-organ involvement, similar to severe disease in falciparum malaria. Severe vivax malaria manifestations can present with cerebral malaria (4), severe anaemia (5), jaundice (5), acute respiratory distress syndrome (6), splenic rupture (7), acute renal failure (8–10), severe thrombocytopenia (7, 11) and pancytopenia (5). The underlying mechanisms of severe manifestations are not well understood, and may be of an inflammatory pathology similar to that seen in falciparum malaria. During pregnancy, infection with *P. vivax*, as with *P. falciparum*, reduces birth weight. In primigravidae, the reduction is approximately two-thirds that associated with *P. falciparum* (110 g

compared to 170 g), but the effect does not decline with successive pregnancies, indeed in the one large series in which this was studied, it increased (12). Chronic anaemia, sequestration and pro-inflammatory cytokines in the placenta lead to lower birth weights (12, 14), increasing the risk of low birth weight (<2500 g) and thus the risk of neonatal death.

## A10.2 Diagnosis

Diagnosis of vivax malaria is based on microscopy. RDTs based on immunochromatographic methods are available for the detection of non-falciparum malaria. However, their sensitivities for detecting parasitaemias of  $\leq 500/\mu\text{l}$  are low (15–21). The relatively high cost of these tests is a further impediment to their widespread use in endemic areas. Molecular markers for genotyping *P. vivax* parasites are available for the *dhfr* gene, and those for chloroquine resistance are under development.

## A10.3 Treatment

The objectives of treatment of vivax malaria are to cure the acute infection and also to clear hypnozoites from the liver to prevent future relapses. This is known as radical cure.

There are relatively few studies on the treatment of *P. vivax*. Only 11% of the 435 published antimalarial drug trials have been on *P. vivax* malaria (22).

### A10.3.1 Standard oral regimen

Chloroquine monotherapy (25 mg base/kg bw over 3 days) is recommended as the standard treatment for vivax malaria because the parasite remains sensitive to chloroquine in much of the world. Primaquine (0.25 or 0.5 mg base/kg bw in a single daily dose for 14 days) is used as a supplement to the standard treatment for the purpose of eradicating dormant parasites in the liver and preventing relapses. The optimal dose of primaquine differs in geographical areas depending on the relapsing nature of the infecting strain, and it remains unclear in patients of heavy body weight (23). This combination of chloroquine and primaquine constitutes treatment to achieve radical cure of vivax malaria.

Primaquine also has weak activity against blood stage parasites. The radical cure regimen of vivax malaria with chloroquine and primaquine therefore conforms to the definition of a combination therapy. The combination of any antimalarial against *P. vivax* infections with primaquine has improved cure rates

(24, 28) and is therefore useful in the treatment of chloroquine-resistant *P. vivax* infections.

### A10.3.2 Treatment of drug-resistant *P. vivax*

Therapeutic efficacy data available to date indicate that *P. vivax* remains sensitive to chloroquine throughout most of the world (26, 29–43). Indonesia is exceptional in that high therapeutic failure rates ranging from 5% to 84% have been reported on day 28 of follow-up (25, 26, 44–49). There are reports of chloroquine failure as both treatment and prophylaxis against *P. vivax* malaria from several countries and regions where the species is endemic (50–53). Some of these studies did not measure chloroquine drug concentrations, so that it is questionable whether these findings represented strictly defined chloroquine resistance (34, 38, 39, 41, 43, 54–57).

Antimalarials that are effective against *P. falciparum* are generally effective against the other human malaras. The exception to this is sulfadoxine-pyrimethamine to which *P. vivax* is commonly resistant. Owing to the high prevalence of *dhfr* mutations (*pvdhfr*) in *P. vivax*, resistance to sulfadoxine-pyrimethamine develops faster in this parasite than in *P. falciparum*, and resistant *P. vivax* become prevalent in areas where this drug is used for the treatment of falciparum malaria (37, 58–66).

The recommended treatment for chloroquine-resistant *P. vivax* is quinine (10 mg salt/kg bw three times a day for 7 days) (67). However, it is not an ideal treatment because of the toxicity of quinine and the poor adherence to this regimen. A study in Thailand has found that treatment of vivax malaria with quinine leads to early relapses. This may be because quinine has a short half-life, and no antihyponozoite activity (37).

Other treatments that have been tested for the treatment of *P. vivax* malaria with varying degrees of efficacy include the following drugs.

Amodiaquine (25–30 mg base/kg bw given over 3 days) has been used effectively for the treatment of chloroquine-resistant vivax malaria (67) and has been well tolerated (68–70). Primaquine must be added for radical cure. Mild nausea, vomiting and abdominal pain are the commonly reported adverse reactions (70).

Mefloquine (15 mg base/kg bw as a single dose) has been found to be highly effective with a treatment success of 100% (37).

Halofantrine (24 mg base/kg bw over 12 hours in three divided doses) has shown varied efficacy in vivax malaria (24, 25, 36, 37) but is not recommended because of its known cardiotoxicity.

Doxycycline alone (100 mg twice a day for 7 days) is not recommended for the treatment of vivax malaria because of its poor efficacy (46).

Artemisinin derivatives as monotherapy for 3–7 days have shown poor efficacy in vivax malaria, with day 28 cure rates of 47–77% (27, 37, 55). The addition of primaquine to these regimes improved the day 28 cure rates to 100% (27, 71).

Combinations of chloroquine (25 mg base/kg bw given in divided doses over 3 days) and sulfadoxine-pyrimethamine (based on a pyrimethamine dosage of 1.25 mg/kg bw as a single dose) or chloroquine (25 mg base/kg bw in divided doses over 3 days) and doxycycline (100 mg twice a day for 7 days) have shown modest efficacy (71–82%) and have not shown significant improvements in cure rate compared with chloroquine alone (46, 70).

Other combinations of artesunate (4 mg/kg bw, single daily dose for 3 days) and sulfadoxine-pyrimethamine (based on a pyrimethamine dosage of 1.25 mg/kg bw, single dose), when used in areas of high chloroquine-resistant *P. vivax* (west Papua), produced 28-day cure rates of less than 90% (64).

Artemether-lumefantrine (the latter formerly known as benflumetol) (16 tablets for 3 days or 20 tablets for 5 days given twice a day in divided doses) has shown significantly shorter parasite clearance times than in the standard regimen of chloroquine + primaquine. However, these regimens were associated with higher relapse rates than with chloroquine + primaquine in the nine months of follow up (72). In another evaluation of the efficacy of artemether-lumefantrine against *P. falciparum* the population studied included patients who were also infected with *P. vivax*. Although high rates of *P. vivax* parasite clearance were noted within 42 h, in 6/16 patients parasites reappeared before 28 days (73).

The best combinations for the treatment of *P. vivax* are those containing primaquine when given at antihypnozoite doses (24, 29, 37, 39, 56, 70, 74, 75). The addition of primaquine at the standard dose of 0.25 mg/kg bw, once daily for 14 days to chloroquine has improved the cure rates in chloroquine-resistant vivax malaria (25, 39, 54, 56). Further, at higher doses (0.5–0.6 mg/kg, once daily for 14 days), primaquine appears to be effective in areas where there are presumed primaquine-resistant hypnozoite infections (27, 76).

Unlike *P. falciparum*, *P. vivax* cannot be cultured continuously *in vitro*, so that it is more difficult to determine the *in vitro* sensitivity of *P. vivax* to antimalarials. *In vivo* assessment of the therapeutic efficacy of drugs against *P. vivax* malaria is also compounded by difficulties in distinguishing recrudescences due to drug-resistant infections from relapses. The interval between the primary and repeat infection can serve as a general guide. If the recurrence appears within 16 days of starting treatment of the primary infection it is almost certainly a recrudescence due to therapeutic failure. A recurrence between days 17 and

28 may be either a recrudescence by chloroquine-resistant parasites or a relapse. Beyond day 28 any recurrence probably represents a relapse in an infection of chloroquine-sensitive *P. vivax* (77, 78). A recurrent vivax parasitaemia in the presence of chloroquine blood levels exceeding 100 ng/ml, and a parasite genotype identical with the primary infection as detected by PCR are more suggestive of chloroquine resistance of the primary infection than a relapse infection.

### A10.3.3 Preventive therapy for relapses

Primaquine is the only available and marketed drug that can eliminate the latent hypnozoite reservoirs of *P. vivax* and *P. ovale* that cause relapses. There is no evidence that treatment courses shorter than 14 days are effective in preventing relapses (39, 56, 79, 80). Relapse rates and primaquine sensitivity vary geographically. The reported incidences of relapses range from 11–26.7% in India (56, 81) to 49–51% in Afghanistan (79). Relapses may occur one to four times after initiation of radical treatment (80, 82). In patients treated with chloroquine, the first relapse is often suppressed by pharmacologically active concentrations of chloroquine and therefore does not manifest clinically or parasitologically. The first clinically manifested relapse has been reported any time after day 16 and up to four years following the primary infection (83, 85). Host immunity is also considered to be a major contributor to the therapeutic response against relapses (86). Risk factors associated with relapses are female sex, higher parasitaemia at baseline, shorter number of days with symptoms prior to baseline, and a lower dose of primaquine (83).

Hypnozoites of many strains of *P. vivax* are susceptible to a total dose of 210 mg of primaquine (24, 37, 54, 75, 79, 83, 87). Infections with the Chesson strain or primaquine-resistant strains prevalent in southern regions of South-East Asia and Oceania require a higher dosage of primaquine (22.5 mg or 30 mg per day for 14 days for a total dose of 315 mg or 420 mg, respectively) to prevent relapses (56, 74, 76). Primaquine is contraindicated in patients with severe variants of the inherited enzyme deficiency, G6PD (88, 89) (see section below on adverse effects).

Although, the long 14-day course of primaquine is a clear disadvantage, it has been shown that poor adherence to unsupervised 14-day primaquine therapy can be overcome effectively through patient education (90). The lengthy treatment courses and follow-up periods make the assessment of primaquine efficacy difficult. Thus, the identification of *P. vivax* strains that are resistant to chloroquine and/or to primaquine presents major challenges.

Alternative drugs are much needed for the radical treatment of *P. vivax* malaria resistant to chloroquine and/or primaquine. A new drug, tafenoquine, is currently

being evaluated as an alternative to primaquine in the prevention of relapses (91). However, this too has haemolytic potential in G6PD-deficient individuals.

#### A10.3.4 Treatment of severe and complicated vivax malaria

Prompt and effective management should be the same as for severe and complicated falciparum malaria (set out in section 8 of the main document).

#### A10.3.5 Treatment of malaria caused by *P. ovale* and *P. malariae*

Resistance of *P. ovale* and *P. malariae* to antimalarials is not well characterized and these infections are considered to be generally sensitive to chloroquine. Only a single study in Indonesia has reported *P. malariae* resistant to chloroquine (63). The recommended treatment for radical cure of *P. ovale*, another relapsing malaria, is the same as that for *P. vivax*, i.e. with chloroquine and primaquine. The high prevalence of G6PD-deficiency status in areas endemic for *P. ovale* calls for the same caution in the use of primaquine as stated in section A10.3.3. *P. malariae* forms no hypnozoites and so does not require radical cure with primaquine.

#### A10.3.6 Adverse effects and contraindications

*Chloroquine* is generally well tolerated. Common adverse effects include mild dizziness, nausea, vomiting, abdominal pain and itching (3, 67 86).

*Primaquine* can induce a life-threatening haemolysis in those who are deficient in the enzyme G6PD (see section A10.3.3). A full course of primaquine, given as a daily dose of 0.25 mg base/kg bw for 14 days, is reported to be safe in populations where G6PD deficiency is either absent or readily diagnosable but could induce a self-limiting haemolysis in those with mild G6PD deficiency (34, 54, 56). To reduce the risk of haemolysis in such individuals, an intermittent primaquine regimen of 0.75 mg base/kg weekly for 8 weeks can be given under medical supervision. This regimen is safe and effective (89). In non-G6PD deficient individuals, a high dose of primaquine (30 mg/day) has been shown to be safe and effective for Chesson strain *P. vivax* malaria in South-East Asia during a 28-day follow-up (27, 74, 76). In regions where prevalence of G6PD deficiency is relatively high, G6PD testing is required before administration of primaquine. Primaquine is not recommended during pregnancy and in infancy since limited safety data are available in these groups (67). Abdominal pain and/or cramps are commonly reported when primaquine is taken on an empty stomach. Gastrointestinal toxicity is dose-related and is improved by taking primaquine with food. Primaquine may cause weakness, uneasiness in the chest, haemolytic anaemia, methaemoglobinaemia (which occurs in non-

haemolysed red cells), leukopenia, and suppression of myeloid series. Therefore, primaquine should not be given in conditions predisposing to granulocytopenia, including rheumatoid arthritis and lupus erythematosus.

## A10.4 Monitoring therapeutic efficacy

There is a need to monitor the antimalarial sensitivity of *P. vivax* in order to improve the treatment of vivax malaria, in particular in view of its emerging resistance to chloroquine. An *in vitro* test system has been developed for assessing the parasite's sensitivity to antimalarials (92, 93). A modified version of the standard WHO *in vitro* microtest for determination of the antimalarial sensitivity of *P. falciparum* has been used successfully for assessing the antimalarial sensitivity of *P. vivax* populations and for screening the efficacy of new antimalarials by measuring minimal inhibitory concentration (MIC), and the concentrations providing 50% and 90% inhibition (IC<sub>50</sub>), and (IC<sub>90</sub>) (87, 89). WHO has also recently introduced a revised protocol for *in vivo* monitoring of the therapeutic efficacy of chloroquine in *P. vivax* malaria (95). The revised protocol includes measurement of blood chloroquine levels, PCR genotyping and the use of molecular markers (only available for the *dhfr* gene) to help clarify and complete the overall picture of drug resistance. A better understanding of the molecular mechanisms underlying drug resistance in *P. vivax* is needed to improve the monitoring of chloroquine resistance.

## A10.5 Conclusions and recommendations

- The standard oral regimen of chloroquine of 25 mg base/kg bw given over 3 days + primaquine at either a low (0.25 mg base/kg bw per day for 14 days) or high (0.5–0.75 mg base/kg bw per day for 14 days) dose is effective and safe for the radical cure of chloroquine-sensitive *P. vivax* malaria in patients with no G6PD deficiency.
- Of the limited alternative treatments that have been evaluated, amodiaquine is a promising monotherapy and has been shown to be effective for the treatment of chloroquine-resistant *P. vivax* malaria (cure rate >90%).
- In areas where infections of drug-resistant *P. falciparum* and/or *P. vivax* are common, drug regimens to treat both species effectively must be used. An ACT that does not include sulfadoxine-pyrimethamine would be a good choice.
- The use of high-dose primaquine (0.5–0.75 mg base/kg bw per day for 14 days), with either chloroquine or another effective antimalarial, is essential

for trying to prevent relapses of primaquine-resistant or primaquine-tolerant *P. vivax*.

- A primaquine regimen of 0.75 mg base/kg bw once per week for 8 weeks is recommended as antirelapse therapy for *P. vivax* and *P. ovale* malaria in patients with mild G6PD deficiency.
- Increased efforts are needed to evaluate alternative treatments for *P. vivax* strains that are resistant to chloroquine. Urgent needs include establishing *in vitro* culture of *P. vivax* to permit the assessment of drug susceptibility, research to improve understanding of the molecular mechanisms of drug resistance, and the development of better tools for genotyping *P. vivax*.

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## A

- Abdominal pain 92, 94, 103, 109, 115, 188, 227, 230
- Acetaminophen 28
- Acidosis 5, 38, 41-42, 52-53, 55-56, 112
- Acquired infections 15, 163
- ACT 1, 9, 17-24, 26, 27, 29-36, 39, 48-50, 61, 64, 65, 68, 70, 71, 83, 99, 101, 137
- Acute
  - failure, renal 5, 38, 48, 52, 53, 55, 66, 128, 225, 232
  - infection 68, 163, 165, 200, 225, 226
  - malaria
    - falciparum 1, 5, 16, 29, 32, 39, 65, 66, 68, 123-128, 179, 194, 195, 204, 205, 207, 222, 225, 226
    - subtertian 222
    - uncomplicated 7, 16, 29, 32, 39, 124-128, 179, 194, 195, 222
    - vivax 5, 65-69, 225, 226, 232, 233, 237, 238
- Adherence 12, 30-32, 70, 71, 155, 157, 163, 165, 227, 229
- Adjunctive treatment 42, 51
- Adverse
  - effects 7, 14, 17, 30, 33, 38, 39, 48, 88, 89, 94, 106-108, 115, 116, 124, 125, 190, 215, 229, 230
  - events 19, 124, 187, 188, 190-193, 214-216
  - reactions 18, 31, 39, 90, 91, 96, 108, 109, 123-125, 190, 200, 202, 214, 227
- Agranulocytosis 89, 91, 103, 108, 115
- Allergic reactions 90, 91, 125
- 4-aminoquinoline(s) 87, 89, 171
- 8-aminoquinolines 63, 64, 102, 135
- Amodiaquine 12, 18, 20-25, 31, 33, 40, 48, 63-65, 89, 122, 123, 160, 172, 173, 188-191, 193-195, 237
  - artesunate 21-25, 48, 64, 65, 123, 191, 193, 195, 222
  - base 20, 24, 25, 30, 65, 89, 227, 231
  - combination 18, 20, 21, 23, 25, 33, 49, 65, 172, 187-191, 194, 195
  - monotherapy 22, 25, 187-190, 231
  - oral 20, 48, 123, 218, 222, 231
  - prophylaxis 89, 123
  - resistance 12, 18, 21-23, 25, 33, 63, 64, 89, 159, 160, 173, 231
- Anaemic heart failure 55
- Antibacterial drugs 238
- Antiemetics 29
- Antifolate 63, 67, 105, 160, 161, 164-166, 179, 181, 194
  - antimalarials 63, 160, 165-167
  - resistance 63, 67, 160, 161, 164-167, 179, 181
- Antimalarial
  - efficacy 1, 2, 7, 13-16, 21, 33, 40, 66, 70, 84, 87, 172-176, 181, 227, 228, 231, 237-239
  - failure 14, 15, 31, 35, 38, 52, 53, 60, 66, 109, 110, 155, 164, 168, 171, 173, 174, 177, 227, 228
  - medicines 1, 12, 13, 27, 30, 34, 35, 37, 39, 46, 84, 122, 141, 160, 200, 201
  - policy 2, 4, 14, 15, 36, 83, 141, 177
  - regimens 12, 27, 30, 39, 163, 165, 179, 231

- resistance 1, 2, 7, 12-18, 23, 26, 30, 31, 37, 41, 42, 63, 66, 67, 137-143, 155-173, 175-182, 226, 227, 230, 231
- toxicity 89, 98, 100, 103, 109, 116, 118, 123, 126, 201, 238
- treatment 1-4, 7-12, 14-17, 29-42, 45-47, 49, 50, 58-63, 66, 67, 69-73, 83, 84, 134-142, 170-182, 200-203, 226-228, 230-232
- Antipyretic
  - drugs 27, 28, 52
  - medicines 1
- Anti-relapse therapy 71
- Antiretrovirals 201, 202
- Artemether 17, 21-24, 26, 37, 38, 44-49, 58-60, 95-97, 100-102, 125, 126, 191-193, 195, 210-212, 218, 220-222, 228
  - benflumetol 96, 101, 125, 142, 195, 228
  - intramuscular 42, 44, 47, 49, 59, 60, 96, 97, 100, 125, 126, 210, 212, 221
  - lumefantrine 21-24, 26, 37, 38, 40, 48, 64, 96, 97, 101, 102, 122, 126, 173, 179, 191-193, 195, 228
    - combination 23, 24, 49, 96, 191, 195
    - regimen 21, 23, 24, 37, 191, 228
    - treatment 23, 24, 26, 27, 37, 38, 40, 41, 48, 49, 64, 102, 126, 173, 179, 191-193, 195, 201, 228
  - quinine 37, 38, 40, 42-49, 58, 59, 64, 101, 122, 210-212, 220, 221
- Artemisinin 17, 18, 30, 31, 33-35, 44-50, 58, 59, 61, 63, 64, 95-100, 124, 125, 134, 135, 139, 161-165, 185, 186, 213, 218-221
  - combination 1, 17, 18, 20, 23, 33, 63, 83, 95, 96, 99, 139, 161, 164, 170, 172, 185-187, 191
  - component 18
  - compounds 17, 18, 30, 97, 124, 125, 171
  - derivatives 1, 12, 17, 30, 31, 33-35, 44-48, 58, 59, 61, 63, 64, 95, 96, 124, 125, 135, 163-165, 185, 186, 218-220
  - dihydro- 17, 44, 47, 95-99, 122, 182
  - non- 18, 185, 186, 191
  - oral 17, 20, 34, 47, 50, 61, 95, 97-99, 101, 124, 125, 204, 219
  - rectal 44, 46, 47, 49, 50, 95, 98, 99, 124, 213, 221
  - resistance 1, 12, 13, 17, 18, 20, 23, 30, 33, 42, 63, 64, 95, 139-141, 161-167, 170, 171, 180, 219
  - suppositories 49, 50, 95, 99
  - susceptibility 63, 161, 162, 165, 170
  - treatment 1, 17, 18, 30, 31, 33-35, 42-50, 57-59, 61-64, 83, 135, 139-141, 149, 186, 187, 190, 191, 213, 218-221
- Artemotil 17, 42, 44, 45, 47, 95-97, 100, 212
- Artesunate 20-26, 32, 34, 36, 37, 44-51, 58-61, 64, 65, 94-99, 125, 126, 142, 165, 166, 191-193, 209, 210, 213, 214, 217-222
  - amodiaquine 21-25, 48, 64, 65, 123, 191, 193, 195, 222
  - combinations 17, 19, 20, 23, 25, 32, 49, 61, 95, 96, 140, 165, 166, 171, 180, 185, 191, 228
  - dose 21-26, 34, 47, 49-51, 61, 93, 97-99, 171, 214, 217, 219, 220, 228, 234
  - formulation 22, 32, 49, 50, 59, 99
  - intramuscular 42, 44, 47, 49, 59, 60, 96-98, 123, 125, 126, 210, 221
  - intrarectal 51, 126, 220, 221
  - intravenous 44, 47, 49, 59, 60, 98, 125, 209, 210, 218, 221

mefloquine 21-23, 25, 26, 49, 61, 93, 94, 123, 137, 140, 142, 165, 166, 171, 180, 192, 193, 217-219, 222  
oral 20, 36, 47, 48, 50, 59-61, 95, 98, 99, 123, 125, 126, 217-219, 222, 234  
parenteral 36, 37, 42, 44, 46-49, 58, 59, 95, 213, 217, 219, 221  
rectal 36, 44, 46, 47, 49, 50, 60, 95, 98, 99, 126, 209, 213, 221  
resistance 17, 21-23, 25, 42, 64, 70, 95, 137, 140, 142, 165, 166, 171, 180, 219, 236  
sulfadoxine-pyrimethamine 21-23, 25, 64, 65, 70, 166, 171, 191, 195, 228, 236, 237  
suppositories 49-51, 59, 95, 99, 214

#### Asymptomatic

malaria 1, 33, 39, 138, 152, 158, 203, 233  
parasitaemia 39

Atovaquone 16, 20, 22, 37, 38, 40, 103-105, 113, 126, 127, 161, 162, 164, 179

combination 16, 20-22, 105, 161, 179

proguanil 16, 20, 22, 37, 38, 40, 103-105, 126, 127, 161, 165

resistance 16, 105, 161, 162, 164, 165, 179

## B

Benign malaria 66, 225

Biguanides 105, 106, 160, 161

#### Blood

chloroquine 1, 16, 42, 63, 67, 94, 118, 119, 134, 171, 175, 176, 182, 226, 227, 229, 231, 236-239

infection 5, 31, 54, 57, 58, 62, 63, 66, 67, 94, 134, 135, 137, 149, 158, 159, 163, 200, 204, 228, 229

parasites

*P. vivax* 5, 148, 226-229

schizontocidal 16

transfusion 42, 55, 56, 58, 215, 221

Breast milk 34, 88, 90, 92, 94, 106-109, 111, 115

## C

Candidate replacement drugs 155

#### Cardiac

arrest 110

failure 110, 215

Cardiotoxicity 37, 38, 89, 110, 126, 227

Cerebral malaria 5, 7, 29, 49, 52, 54, 57, 66, 120, 121, 125, 129, 203, 211, 212, 214, 220, 221

Chloroquine 12, 13, 63-67, 70, 87-89, 118, 119, 122, 123, 128, 129, 139-142, 158-160, 162-173, 175, 176, 180-182, 186, 187, 193-195, 226-239

base 20, 30, 63, 65, 70, 87, 89, 226-228, 230-232

blood 16, 42, 63, 67, 95, 119, 134, 158, 163, 171, 175, 176, 182, 226, 229, 238, 239

combined 65, 70, 123, 170, 193, 195, 237

doxycycline 20, 40, 170, 228, 235

failure

treatment 1, 35, 168, 171-173, 182, 186, 222, 227-229, 236

oral 20, 63, 119, 122, 123, 222, 226, 231, 234

- parenteral 42, 43, 88
- resistance
  - P. falciparum* 12, 19, 25, 63, 64, 66, 68, 87, 139, 140, 155, 159, 160, 166-169, 172, 175, 180, 182, 183
  - P. vivax* 12, 13, 62-64, 66-68, 70, 176, 226, 227, 229-232, 234-236
- sensitive 13, 63, 65, 66, 70, 138-140, 158, 163, 226, 227, 229-231, 234
- sulfadoxine-pyrimethamine 1, 12, 16, 18, 25, 63-65, 90, 141, 142, 166-169, 171-173, 180-182, 186-188, 194, 195, 228, 236, 237
- Chlorproguanil 16, 21, 33, 63, 106, 107, 127, 160, 179, 182
- Clindamycin 17, 20, 32-34, 37, 38, 40, 48, 49, 53, 114, 115, 122, 170
- Clinical
  - diagnosis 8, 9, 11, 27, 46, 54, 62, 69, 84, 145, 147, 150-152, 173, 182, 204, 233
  - disease 5, 8, 69, 84, 133, 147, 201, 204, 237
  - failure 5, 14, 35, 48, 52, 128, 155, 168, 171, 173, 174, 186, 187, 190, 200, 232
  - malaria 5, 6, 8, 11, 12, 44-46, 48-54, 84, 122-129, 133, 147, 148, 150-152, 172-176, 199-201, 219-221, 232-234, 236-238
- Co-formulation 30, 32, 104, 105
- Co-infection 11, 203, 204
- Combinations
  - aminoquinoline-antifolate 194
  - amodiaquine 18, 20, 21, 23-25, 33, 48, 65, 172, 188-191, 193-195
  - artemether-lumefantrine 23, 24, 48, 191, 193, 195, 228
  - artemisinin-based, non artemisinin-based 1, 17, 18, 20, 23, 33, 61, 63, 83, 95, 96, 99, 139, 164, 165, 170, 185, 186, 191
  - artesunate-mefloquine 142, 165, 180
  - atovaquone-proguanil 16, 20, 22, 161
  - chemotherapy 1, 128, 142, 178, 179
  - chloroquine-doxycycline 20, 32, 128, 170, 228, 235
  - drug 16-18, 21, 49, 63, 96, 99, 106, 128, 139, 140, 142, 159, 164-166, 170-172, 175, 176, 178-180
  - fixed-dose 61, 90, 92, 99
  - mefloquine 20, 23, 25, 33, 49, 61, 63, 110, 137, 140, 142, 165, 166, 171, 179, 180
  - RDT 11
  - sulfadoxine-pyrimethamine, amodiaquine 172, 195
  - sulfone-biguanide 161
  - treatment 1, 16, 17, 19, 20, 22-25, 33, 34, 48, 49, 61, 63, 65, 66, 83, 92, 137, 138, 186-191, 193-195, 226-228
- Complex emergencies 11, 69-71
- Complicated
  - malaria
    - falciparum 1, 66, 194, 195, 230
    - vivax 65, 66, 229, 230
- Confirmatory diagnosis 8, 9, 149, 150
- Convulsions 29, 41, 52, 53, 57, 88, 89, 91, 92, 94, 124, 214-216
- Corticosteroids 52, 54, 55

Cotrimoxazole 25, 39, 93, 104, 200-202  
Cycloguanil 105, 106, 127, 160, 161  
    resistance 105, 160, 161

## D

Dapsone 16, 20, 21, 33-35, 91, 92, 106-108, 179, 182

### *De novo*

    mutation 162, 163, 166  
    resistance 1, 17, 157-159, 162-166

Dexamethasone 55, 214, 215, 221

Diagnosis 8-11, 26, 27, 36, 46, 50, 54, 60, 62, 69, 84, 147, 149-152, 182, 226, 232, 233  
    clinical 8, 9, 11, 27, 46, 54, 62, 69, 84, 147, 150-152, 172, 181, 204, 233, 237  
    parasitological 8-11, 84, 150, 152, 172, 201  
    rapid 9, 46, 62, 149-152, 233

Dihydroartemisinin 17, 22, 47, 95-99, 122, 125, 126, 214

Doxycycline 20, 32, 37, 38, 40, 48, 49, 111-114, 122, 128, 129, 170, 228, 235

### Drug

    combinations 16, 18, 165, 172, 176, 180  
    efficacy 7, 13, 14, 16, 18, 21, 40, 58, 84, 87, 117, 123, 172-177, 181, 227-229, 237-239  
    hepatotoxic 113  
    interactions 9, 88, 89, 93, 94, 96-100, 102-104, 106-108, 110, 112, 114, 115, 122, 129, 156, 201, 202  
    intramuscular 40, 42, 52, 59, 60, 90, 96-98, 100, 114, 116, 118, 123  
    intrarectal 40, 118  
    oral 17, 19, 27, 28, 48, 59-61, 63, 95, 97-99, 101, 104-106, 113-115, 118, 119, 122-125, 127, 128, 218, 219  
    patterns 2, 14, 155, 157, 172  
    resistance 1, 2, 7, 12-18, 26, 31, 42, 63, 70, 73, 138-143, 155-173, 175-182, 217, 226, 227, 231, 232  
    resistant 17, 89, 101, 125, 126, 138-140, 142, 156-160, 162-166, 175-178, 180-182, 222, 227-229, 231, 232, 235, 237

## E

Epidemic 2, 6, 11, 59, 60, 69-72, 84, 155, 176, 182, 199, 202, 203, 236

    falciparum 11, 59, 60, 69, 70, 84, 155, 182, 203, 236

    mixed 69, 70

    vivax 11, 68-71, 155, 236, 237

## F

### Failure

    acute renal 5, 38, 48, 52, 53, 55, 66, 128, 225, 232

    anaemic heart 55

    antimalarial treatment 14, 15, 31, 35, 38, 52, 53, 177, 200

    blood 14, 24, 27, 31, 52, 53, 55, 110, 139, 171, 173, 215, 217, 229

    cardiac 110, 215

- chloroquine 1, 35, 166-168, 171, 173, 182, 186, 222, 227, 229, 235, 236
- clinical 5, 14, 35, 48, 53, 127, 128, 155, 164, 168, 171, 173-175, 186, 187, 189, 200, 232
- impairment 56, 112, 114
- late
  - clinical 173, 174
  - parasitological 173-175
- parasite 5, 9, 14, 18, 38, 60, 121, 163, 173, 185, 217, 218, 222, 229
- parasitological 9, 14, 17, 21, 31, 173-175, 185, 187, 190, 191
- renal 5, 38, 48, 52, 53, 55, 56, 66, 109, 110, 112, 114, 128, 225, 232
- total 15, 20, 24, 174, 178, 185, 186
- treatment 5-7, 14, 15, 17-20, 31, 32, 34, 35, 38, 39, 48, 155, 156, 167, 168, 171-175, 177, 178, 186, 187, 189-191, 217-219, 226-229
- Falciparum 31-41, 55-66, 68-70, 123-128, 135-137, 139-142, 157-162, 166-170, 178-183, 193-195, 203-205, 207-211, 219-222, 225-228, 233-237
  - chloroquine 12, 42, 63-67, 70, 87-89, 128, 139-142, 158-160, 166-169, 171, 172, 175, 180-182, 194, 195, 226-231, 233-237
  - infections 5, 11, 12, 38, 39, 62-66, 68-70, 87, 134, 135, 137, 138, 141, 142, 157, 158, 175, 200, 203, 204, 225, 226, 228
  - malaria 16, 17, 27-29, 31-43, 55-66, 68-70, 123-128, 139-142, 151, 152, 178-183, 193-195, 199, 200, 203-205, 207-217, 219-222, 225-239
    - complicated 66, 194, 195, 230
    - non-, un- 62, 226
  - parasites 5, 11, 15-17, 56, 60, 62, 87, 108, 109, 135-142, 148, 149, 157-160, 172, 207-210, 216-219, 225-228
  - Plasmodium 5, 95, 123-128, 136, 141, 142, 148, 151, 152, 167-170, 178-182, 193-195, 203, 204, 219-222, 225, 233-237, 239
  - resistance 1, 2, 12, 16-19, 22, 23, 25, 26, 63, 64, 66, 67, 139-142, 155, 157-162, 166-173, 175, 178-182, 226, 227, 234-236
    - mefloquine 12, 22, 23, 63, 64, 124, 137, 140, 142, 160, 161, 166, 167, 169-171, 179-181, 218, 219, 236
- First-line treatment 15, 20, 31, 33, 36, 38, 65, 172, 178

## G

- G6PD deficiency 65, 71, 103, 230-232
- Gametocytocidal 70, 71, 102, 134, 135, 137, 139, 141
- Glasgow coma scale 42
- Guidelines 1-4, 6, 8, 10, 12, 14-16, 20-22, 28-30, 32, 38-40, 72, 73, 75, 77-81, 83, 84, 176-178

## H

- Halofantrine 22, 33, 37, 38, 88, 94, 101, 110, 162, 169, 179, 201, 227, 233, 234, 237
- Hepatic
  - dysfunction 40, 48
  - impairments 97, 98, 114
  - schizonts 16, 71, 91, 95, 102, 105, 175
- Hepatitis 89, 91, 108, 215, 216

Hepatotoxic 113

HIV 2, 10, 38, 39, 73, 75, 147, 164, 197, 199-204, 215, 216

AIDS 10, 38, 39, 73, 75, 147, 197, 199-204

co-infection 203, 204

infected 10, 39, 200-202, 204

infection 38, 39, 164, 199-204

patients 10, 38, 39, 201-204

## I

Infants 33-36, 69, 112, 116-118, 128, 203

Infection(s)

acquired 12, 14, 15, 64, 158, 163, 165, 203

acute 5, 38, 66, 68, 163, 165, 173, 200, 225, 226, 232

bacterial 54, 57, 58, 111, 115

blood 5, 31, 53, 57, 58, 62, 63, 67, 94, 134, 135, 137, 149, 158, 159, 162, 173, 175, 200

chloroquine 1, 12, 63-67, 70, 87, 134, 139, 142, 158, 173, 175, 186, 226-231, 234-237

co- 11, 203, 204

HIV, HIV/AIDS 38, 39, 73, 75, 164, 199-204

liver 62, 64, 94, 163, 225, 226

malaria 5-7, 10-12, 14-18, 36-40, 57, 58, 62-70, 75, 134-143, 147, 149-151, 157-159, 163-166, 199-204, 225-228, 230-237

mixed 68-70, 142, 150

opportunistic 39, 200-202

placental 200, 203

*Plasmodium*

*falciparum* 5, 127, 136, 137, 142, 151, 178, 203, 204, 219, 222, 225, 233-236

*ovale* 5, 62, 223, 225, 227, 229, 231, 233, 235

*vivax* 5, 62, 223, 225-229, 231-237

primaquine 11, 63-66, 68, 70, 135, 137, 226, 228-232, 235, 236

primary 7, 12, 62, 138, 157, 158, 163, 164, 218, 225, 228, 229

recrudescence 15, 142, 158, 163, 173, 218

recurrent 20, 58, 173, 175, 229

relapse 11, 62, 64, 66, 175, 225, 229, 236

repeated 62, 64, 225

resistant 17, 39, 64, 65, 67, 138, 139, 142, 158-160, 163-165, 175, 177, 178, 222, 227-232, 237

*Salmonella* 54, 57

single 62, 65, 164, 230

transmissible 157, 158

Insecticide-treated nets 69, 134, 140

Intermittent

regimen 230

treatment 1, 2, 22, 23, 39, 40, 200-202

## K

Kwashiorkor 117-120, 128, 129

**L**

Lactating women 34, 35, 112, 114, 127

Lumefantrine 20-24, 26, 33, 37, 38, 40, 48, 64, 96, 97, 101, 102, 110, 122, 126, 191-193, 195, 228

**M****Malaria**

acute 5, 6, 16, 26, 29, 38, 52, 55, 56, 66, 68, 101, 123-128, 163, 194, 195, 225, 226, 232

anaemia 5, 8, 12, 14, 32, 41, 55, 56, 58, 66, 103, 107, 108, 110, 121, 215, 225, 226

asymptomatic 33, 39, 137, 152, 158, 203, 233

benign 66, 225, 232

blood 5, 6, 14, 16, 42, 52-59, 62, 63, 67, 71, 94, 95, 102, 133-135, 148, 149, 158, 159, 172-173, 215-217

cerebral, adult 203, 214

chemotherapy 1, 123, 125-128, 141, 142, 178, 179, 182, 221, 234, 236, 237, 239

clinical 1, 2, 5, 6, 8, 11, 12, 35-37, 41, 42, 44-46, 50-54, 122-129, 147, 150-152, 172-177, 179, 200, 201, 232-234

complicated / non-complicated 194, *see also*: malaria, uncomplicated

diagnosis 8-11, 26, 27, 36, 46, 50, 54, 60, 62, 69, 84, 147, 149-152, 182, 226, 232, 233

epidemics 2, 6, 11, 69-71, 155, 176, 182, 199, 202

frequent 39, 46, 56, 59, 64, 89, 97, 170, 190, 193, 199

human 5, 62, 91, 127, 133, 134, 148, 151, 203, 204, 225, 227, 233, 235, 239

ICT 151, 152, 233

imported 151

incidence 6, 10, 12, 26, 49, 61, 133, 134, 137, 138, 140, 142, 147, 165, 180, 204, 225

microscopy 9-11, 62, 68, 147-152, 226, 233

moderate 10, 11, 60, 65, 126, 137, 159, 173, 174, 192, 213, 221

ovale 5, 11, 37, 62, 63, 65-69, 73, 75, 87, 102, 108, 135, 148, 223, 225, 229-233

parasites 5, 6, 8, 9, 11-18, 60, 62, 133-142, 147, 148, 150, 151, 155-159, 163-166, 172, 173, 175, 176, 207-212, 216-219, 225-228

placental 200, 203

*plasmodium*

*falciparum* 123-128, 136, 137, 141, 142, 151, 152, 167, 178-183, 185, 193-195, 203-205, 207, 209-211, 217, 219-222, 225, 233-237

non-falciparum 62, 226

uncomplicated 2, 5, 7, 8, 16-17, 27, 29, 34, 37-37, 50, 60-62, 70, 84, 96, 109, 124, 125, 142, 179, 181, 183, 185, 187, 189, 191, 193-195, 216, 222, 235, 236, 239

*vivax* 5, 62, 148, 152, 182, 223, 225-229, 231-239

rapid diagnostic tests 9, 62, 69, 148, 151

## severe

anaemia 5, 8, 41, 55, 56, 58, 66, 110, 215, 225

malaria 1, 2, 5-8, 11, 12, 16, 29, 35-63, 65, 66, 69, 70, 109, 110, 121-128, 147, 200-205, 207-222, 225, 226, 232, 233

non-severe 123

species 5, 11, 18, 62, 63, 66, 68-70, 84, 95, 103, 141, 147, 148, 155, 225, 227, 231

stable / unstable 5, 6, 10, 11, 56, 66, 133, 199-204, 215, 218

subtertian 222

tertian 232

transmission

high 5-8, 10, 11, 14, 27, 32, 33, 55, 56, 60, 66, 68-71, 133, 134, 137-140, 149, 158, 163-165, 203, 204

low 6, 10-12, 14, 32, 45, 46, 56, 58-60, 62, 64, 68, 133, 137-141, 158, 172-174, 217

moderate 10, 11, 14, 60, 137, 173, 174

stable 5, 10, 11, 56, 66, 133, 215, 218

treatment

guidelines 1-4, 6, 8, 10, 14-16, 20-22, 28-30, 32, 38-40, 72, 73, 75, 77, 78, 80, 81, 83, 84, 176-178

policy 2, 4, 14, 15, 83, 141, 174, 177, 204

uncomplicated 1, 2, 7, 8, 16, 17, 23, 27-29, 31-41, 60-63, 70, 124-128, 142, 179, 181, 193-195, 222, 235, 236

Malnutrition 40, 117-121, 128, 129

Mass treatment 71, 72, 168

Mefloquine 20-23, 25, 26, 32, 33, 61, 63, 64, 93, 94, 122-124, 140, 160-163, 165-167, 169-171, 179-181, 192, 193, 217-219, 222

artesunate 21-23, 25, 26, 48, 61, 64, 94, 123, 137, 140, 142, 165, 166, 171, 192, 193, 217-219, 222

combination 20, 23, 25, 33, 49, 63, 110, 137, 140, 142, 165, 166, 171, 179, 180

concentrations 88, 93, 94, 110, 162, 163, 165

monotherapy 22, 169-171

resistance 12, 21-23, 25, 33, 63, 64, 124, 137, 140, 142, 158, 160-171, 176, 179-181, 217-219, 236

treatment 25, 26, 31, 32, 40, 41, 48, 49, 61, 63, 64, 94, 95, 124, 137, 138, 140, 166-168, 192, 193, 217-220, 222, 223, 227, 228

Microscopy 9-11, 31, 62, 68, 147-152, 226, 233

Mixed

epidemics 69, 70

falciparum 68-70

infections 68-70, 142, 143, 150

vivax 67-70

Monitoring 15, 27, 29, 36, 37, 46, 59, 66, 78, 152, 155, 172, 176, 177, 181, 201, 202, 231

Monotherapies 16, 17, 19, 21, 22, 24-27, 34, 37, 139, 169-171, 185-190, 194, 226, 228, 231

Multidrug resistance 23, 171, 217, 218, 237

Mutation 126, 157, 159-164, 166, 169, 170, 172, 176, 179, 180, 182, 227, 236, 237

de novo 162, 163, 165, 166

parasite 156, 159, 160, 162, 166, 172, 176, 227

## N

Nephrotoxic 113

Neurological

sequelae 208, 211, 212

syndrome 124

Neurotoxicity 31, 96, 97, 125

**O**

## Oral

- amodiaquine 20, 48, 123, 218, 222, 231
- artemisinin 17, 18, 20, 34, 47, 49, 50, 61, 95-100, 124, 125, 204, 219
- artesunate 20, 36, 47-50, 59-61, 95, 98, 99, 123, 125, 126, 217-219, 222, 234
- chloroquine 1, 20, 63, 119, 122, 123, 222, 226, 231, 234
- drugs 17, 18, 27, 28, 49, 60, 61, 63, 70, 71, 97-101, 104-106, 113-115, 118, 119, 122, 123, 127, 128, 157, 218, 219, 231
- quinine 34, 47-49, 59, 101, 109, 119, 123, 124, 127, 128, 204, 217, 219, 220
- sulfadoxine-pyrimethamine 20, 39
- therapy 20, 48, 49, 59, 95, 99, 124, 127, 217-219, 222, 234

**P**

- Paracetamol 28, 104
- Parasitaemia 9, 10, 14, 21, 27, 31, 41, 42, 60, 61, 138, 139, 149, 173-175, 192, 203, 204, 214, 217-219, 229
  - asexual 17, 41, 138, 139
  - asymptomatic 39
  - baseline 214, 229
  - hyper- 222
  - malarial 11, 155
  - persistent 175
  - recurrent 173, 229
- Parasitological
  - cure rates 12, 14, 21, 189, 190
  - diagnosis 8-11, 84, 150-152, 173, 201
  - failure 14, 17, 21, 31, 173, 174, 185, 187, 190, 191
- PfCRT 87, 139, 159, 160, 176
- PfHRP 149, 151
- PfMDR 87, 139, 160, 161, 169, 170, 176, 179
- Pharmacovigilance 25, 30, 128, 201, 202
- Phenobarbital 57, 114, 216
- Placental 200, 203
- Plasmodium 5, 62, 95, 103, 123-128, 136, 141, 142, 151, 152, 167-171, 178-182, 193-195, 203, 204, 219-222, 225, 231-239
  - blood stage 62, 226, 236
  - falciparum 123-128, 136, 137, 141, 142, 151, 152, 167-170, 178-183, 185, 193-195, 203-205, 207, 209-211, 217, 219-222, 225, 233-237
  - malariae 5, 62, 148, 167, 171, 223, 225, 227, 229, 231, 233, 235
  - ovale 5, 62, 148, 223, 225, 227, 229, 231, 233, 235
  - resistance 141-143, 167-172, 178-182, 219, 229, 231, 232, 234-236, 238
  - severe 125, 193, 203, 205, 207, 209-211, 213, 215-217, 219-222, 232
  - vivax 5, 62, 148, 152, 171, 182, 223, 225-229, 231-239
- Pregnancy 2, 6, 7, 10, 11, 31-34, 48, 58-60, 62, 96, 104-106, 110, 112, 125-127, 200-203, 225, 226, 232, 233

- Pre-referral 46, 49-51, 84, 213
- Primaquine 11, 33, 40, 63-66, 68, 70, 71, 95, 102, 103, 122, 126, 135, 137, 141, 142, 226-238
  - base 63-65, 70, 71, 102, 226-228, 230-232
  - infection 11, 63, 64, 66, 68, 137, 226, 228, 229
  - intermittent 230
  - regimens 64, 66, 226, 228, 230-232, 234-237
  - resistant 64, 65, 227-232, 234, 236, 237, 239
  - tolerant 232
- Proguanil 16, 20, 22, 33, 37, 38, 40, 63, 103-107, 126, 127, 160, 161, 165, 176
- Protein-energy 118, 119, 121, 128
- Pulmonary 5, 41, 52, 53, 55, 58, 66, 91
  - oedema 5, 41, 53, 55, 58, 66
- Pyrimethamine 20-23, 25, 33, 39, 40, 63-65, 90-93, 160-162, 166-169, 171-173, 178-182, 186-189, 191, 194, 195, 228, 236, 237
  - dose 21-23, 25, 26, 63, 90, 92, 129, 171, 187, 191, 228, 231
  - resistance 1, 12, 13, 16, 18, 21-23, 25, 33, 63, 64, 70, 160-162, 166-171, 173, 176, 178-182, 236
  - sulfadoxine 12, 20-23, 25, 30, 31, 39, 40, 63-65, 90-92, 141, 142, 166-169, 171-173, 179-182, 186-189, 191, 194, 195, 236, 237

## Q

- Quinidine 37, 42, 46, 48, 102, 108, 110, 128
- Quinine 12, 32-34, 37, 38, 42-49, 51, 58, 59, 93, 94, 108-110, 119-124, 127-129, 169, 170, 181, 207-214, 219-221, 227
  - base 47, 51, 93, 109, 120, 207, 227
  - combined 123, 124, 170, 212
  - injection 43, 44, 46, 47, 109, 110, 120, 210, 214
  - intrarectal 51, 209, 220, 221
  - intravenous 43, 44, 47, 49, 59, 110, 111, 120, 127, 208-210, 212, 213, 218, 220, 221
  - monotherapy 34, 170
  - oral 34, 47, 48, 50, 59, 60, 101, 109, 119, 123, 124, 127, 128, 204, 217-219
  - parenteral 37, 42-44, 46-49, 58, 59, 127, 213, 217, 219, 221
  - poisoning 128
  - rectal 44, 46-50, 60, 124, 209, 213, 221
  - resistance 12, 33, 42, 64, 122, 163, 169, 170, 179-181, 227
  - urine 109, 127

## R

- Radical cure 62, 64-66, 87, 102, 226, 227, 230, 231, 233, 237, 238
- Rapid diagnostic tests 9, 62, 69, 148, 151
- Recommendation
  - base 1, 2, 20, 47, 65, 70, 231
  - dosage 123
  - dose 21, 22, 26, 34, 37, 38, 43, 49, 50, 61, 65, 70, 119, 122, 171, 231
  - evidence-based 2, 56
  - key 77, 202

- policy 2, 4, 15, 83  
 treatment 1-4, 8, 10, 11, 14, 15, 17-23, 26-29, 32-41, 43, 44, 46, 48-50, 57, 59-61, 70, 77, 78, 83, 84
- Rectal  
 artemisinin 44, 46, 47, 49, 50, 95, 96, 98, 99, 124, 125, 213, 221  
 artesunate 36, 44, 46, 47, 49-51, 59, 60, 95, 98, 99, 126, 209, 213, 221  
 diazepam 52, 53  
 dihydroartemisinin 47, 95, 98, 99, 126  
 quinine 43-49, 59, 124, 209, 213, 221
- Renal  
 blood flow 119  
 failure 5, 38, 48, 52, 53, 55, 66, 109, 110, 112, 114, 128, 225, 232  
 impairment 41, 53, 56, 97, 98, 106, 112, 114, 115
- Resistance 1-3, 12-23, 25, 26, 30, 31, 41, 42, 63-67, 69, 70, 124-126, 137-143, 155-173, 175-182, 194, 195, 217-219, 226-232, 234-239  
 aminoquinoline 87, 89, 160  
 antibiotic 157, 170, 178  
 antifolate 63, 67, 160, 161, 164-166, 179, 181  
 antimalarial 1, 2, 12-18, 30, 31, 41, 42, 66, 67, 84, 85, 87, 89, 91, 92, 137-140, 155-167, 171, 172, 175-182, 226, 231  
 artemisinin 1, 12, 17, 18, 20, 23, 30, 33, 63, 64, 95, 124, 125, 139, 161-165, 170, 185, 218, 219  
 atovaquone 16, 105, 126, 161, 162, 164, 179  
 chloroquine 12, 13, 42, 63-67, 70, 87, 89, 122, 134, 139, 140, 158-160, 162-173, 175, 176, 180-182, 194, 226-239  
 cycloguanil 105, 160, 161  
 de novo 17, 157-159, 162-166  
 falciparum 1, 12, 16, 17, 23, 63-66, 124-126, 139-142, 157-162, 166-170, 178-183, 194, 195, 222, 226-228, 230, 231, 234-237  
 in vivo 13, 14, 63, 66, 67, 95, 123, 139, 161, 162, 172, 175-177, 179, 180, 228, 231, 234, 235  
 mefloquine 12, 21-23, 25, 33, 63, 64, 124, 137, 140, 142, 160-163, 165-167, 169-171, 179-181, 217-219, 222  
 multidrug 23, 101, 125, 126, 169, 171, 195, 217, 218, 222, 237, 239  
 primaquine 33, 63-66, 70, 71, 95, 141, 142, 226-234, 236-238  
 significant 14, 18, 19, 63, 137, 140-142, 164, 165, 167, 170, 173, 228  
 stable 66, 164  
 sulfadoxine-pyrimethamine 1, 12, 16, 18, 21-23, 25, 39, 63-65, 70, 134, 166-169, 171-173, 180-182, 194, 195, 236, 237  
 sulfone 161  
 suspected 11, 26, 70, 150
- Respiratory distress 38, 41, 42, 55, 66, 215, 216, 225, 232
- Rifampicin 108, 110, 114, 128
- S**
- Second-line treatment 31, 32
- Semi-immune 26, 27, 216

Sequelae 32, 208, 211, 212

neurological 208, 211, 212

Sulfadoxine 12, 20-23, 25, 30, 31, 39, 40, 63-65, 90-92, 141, 142, 166-169, 171-173, 179-182, 186-189, 191, 194, 195, 236, 237

artemisinin 1, 12, 18, 20, 23, 30, 31, 33, 35, 40, 42, 63, 64, 167, 172, 185, 186, 191

artesunate 21-23, 25, 26, 42, 64, 65, 70, 142, 166, 171, 191, 195, 228, 237

intramuscular 42, 43, 90, 92, 123

pyrimethamine 20-23, 25, 33, 39, 40, 63-65, 90-92, 141, 142, 166-169, 171-173, 179-182, 186-189, 191, 194, 195, 228, 236, 237

artesunate 21-23, 25, 26, 42, 64, 65, 70, 123, 142, 166, 171, 191, 195, 228, 237

chloroquine 12, 13, 42, 63-65, 70, 123, 128, 129, 141, 142, 166, 167, 171-173, 180-182, 186, 187, 194, 195, 228, 231, 236, 237

combination 1, 16, 18, 20, 21, 23, 25, 33, 65, 90-92, 166, 171, 172, 185-189, 191, 194, 195

oral 20, 21, 39, 90, 92, 123

resistance 1, 12, 13, 16, 18, 21-23, 25, 33, 42, 63, 64, 70, 166-171, 173, 176, 179-182, 236

resistance 1, 12, 13, 16, 18, 21-23, 25, 33, 42, 63, 64, 70, 166-171, 173, 176, 179-182, 236

## T

Tetracyclines 17, 20, 32-35, 40, 48, 94, 104, 111-114, 121, 129, 170

Thrombocytopenia 66, 91, 92, 94, 110, 112, 115, 225, 232

Toxicity 88-90, 92, 94, 96-101, 103, 104, 106-110, 112, 114-116, 118, 123, 126, 127, 201, 227, 230, 238

Travellers 2, 36-38, 94

## Treatment

adjunctive 42, 51

amodiaquine 12, 18, 20, 22-25, 31, 33, 40, 63-65, 89, 123, 160, 172, 173, 188-191, 193-195, 237

artemether-lumefantrine 23, 24, 26, 37, 38, 40, 48, 64, 102, 126, 173, 179, 191-193, 195, 201, 228

artemisinin(-based) 17, 18, 30, 31, 33-35, 44-50, 58, 59, 61, 63, 64, 95-100, 124, 125, 161, 170, 185, 186, 191, 213, 218-221

chloroquine 1, 12, 13, 42, 63-67, 70, 87-89, 122, 123, 140-142, 158-160, 166-169, 171-173, 180-182, 186, 187, 193-195, 226-239

combination 1, 11, 16-25, 33, 49, 63, 92, 105-107, 140, 142, 164-166, 179, 180, 185-191, 194, 195, 226

failure 14, 15, 19, 20, 31, 38, 48, 52, 110, 155, 173, 174, 177, 178, 185-187, 190, 191, 217-219, 222, 227, 228

first-line 15, 20, 31, 33, 36, 38, 65, 172, 178

generic 83

guidelines 1-4, 6, 8, 10, 14-16, 20-22, 28-30, 32, 38-40, 72, 73, 75, 77, 78, 80, 81, 83, 84, 176-178

hyperparasitaemic 60, 61, 157, 216-219, 222

mass 71, 72, 168

mefloquine 25, 26, 49, 61, 63, 64, 93, 94, 123, 124, 137, 140, 160, 161, 165-167, 169, 170, 179-181, 192, 193, 217-219, 222

oral 17, 19-21, 27, 28, 34, 36, 39, 48, 50, 59-61, 104-107, 114, 115, 121, 122, 124-126, 217-219, 222

parasitological (late) 173-175  
 parenteral 16, 27, 29, 36, 37, 42-44, 46, 48, 49, 52, 58, 59, 61, 88, 92, 115, 216, 217, 219  
*Plasmodium* 5, 95, 103, 123-125, 136, 141, 142, 151, 152, 167-171, 179-182, 187, 189, 193-195, 204, 219-222, 231-239  
     *falciparum* 5, 123-126, 136, 137, 141, 142, 151, 152, 167-170, 179-182, 193-195, 203-205, 207, 209-211, 217, 219-222, 233-237, 239  
     *vivax* 5, 152, 171, 182, 223, 225-229, 231-239  
 policy 2, 4, 14, 15, 83, 141, 174, 177, 204  
 preventive 2, 22, 39, 200-202, 229  
 primaquine 11, 33, 40, 63-66, 68, 70, 71, 95, 126, 135, 137, 141, 142, 226-238  
 quinine 12, 32-34, 37, 38, 42-49, 51, 58, 59, 93, 94, 108-110, 119-121, 127, 128, 169, 170, 181, 207-214, 219-221, 227  
 recommendations 1-4, 10, 11, 14, 15, 19, 20, 22, 23, 26-28, 32-40, 43-46, 49, 50, 57, 59-61, 65, 70, 77, 78, 83, 84  
 regimens 18, 26, 27, 30, 39, 43, 49, 55, 61, 157, 179, 194, 207, 219, 220, 228, 236, 237  
 second-line 31, 32, 139  
 sulfadoxine-pyrimethamine 1, 16, 22, 23, 25, 30, 31, 39, 40, 64, 65, 121, 167-169, 171-173, 180-182, 186-189, 191, 194, 195, 236, 237

## U

Urine 53, 56, 89-91, 106, 107, 109, 112, 114, 115, 127  
     quinine 109, 127, 128  
     urinary tract 89, 90, 113

## V

Vivax 5, 11-13, 37, 62-71, 73, 75, 87, 102, 108, 135, 160, 161, 171, 175, 176, 182, 225-239  
     complicated 66, 230  
     DHPS 161  
     epidemics 11, 69-71, 155  
     infection 5, 11, 12, 62-64, 66-68, 135, 225, 226, 228, 229  
     malaria 5, 10-12, 14, 37, 62-71, 73, 75, 77, 102, 148, 152, 167, 181, 182, 222, 225-239  
     mixed 68-70  
     relapses 62, 64, 226-230, 232, 238  
     resistant 64, 65, 67, 160, 161, 176, 182, 222, 227-232, 234-237, 239  
     sensitive 13, 63, 65, 66, 70, 226, 227, 229-231, 234