# Malaria

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## Summary

- Malaria is very common; a very important cause of mortality and morbidity in the tropics
- Five parasites: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and since 2007; *P. knowlesi* (in Southeast Asia)
- Transmission via female Anopheles mosquitoes which bite at night
- Symptoms: fever and body-ache; sometimes atypical or "chronic" (anaemia, splenomegaly)
- Risk of complicated presentations, mainly with P. falciparum (severe anaemia, kidney failure, cerebral malaria)
- Infections often asymptomatic in semi-immune people (generally low parasitaemia)
- Clinical diagnosis not reliable.
- Often clinical over-diagnosis of malaria and under-diagnosis of other disorders in endemic areas
- Diagnosis via thick smear, thin smear, rapid antigen-detection, DNA-based methods
- Treatment of *P. malariae*: chloroquine
- Treatment of *P. vivax* and *P. ovale:* chloroquine or if possible with primaquine (hypnozoites, G6PD).
- Resistance of *P. vivax* to chloroquine is rising in several areas
- Increasing multidrug resistance of *P. falciparum,* including resistance to artemisinin derivatives
- Combination treatment of *P. falciparum* infection is strongly advised:
    - a) ACT: artemisinin combination treatment (e.g. artemether + lumefantrine; = Co-Artem, Riamet).
    - b) Quinine + (doxycycline or clindamycin)
    - c) Atovaquone + proguanil (Malarone)

- Individual prevention via pyrethroid-impregnated bed net ± chemoprophylaxis; stand-by emergency treatment (self-medication) for certain travelers?
- Population protection via vector control, but increasing resistance of mosquitoes to various insecticides
- First malaria vaccine undergoing phase 4 studies in children of endemic countries

# Malaria in humans

Malaria is the common name for diseases caused by infection with single-celled parasites of the genus Plasmodium. Among the parasites of the genus Plasmodium five species have been identified which regularly cause disease in humans:

- Plasmodium falciparum
- Plasmodium vivax
- Plasmodium ovale
- Plasmodium malariae
- Plasmodium knowlesi

However, in 2017 several malaria outbreaks in Brazil were caused by *P. simium,* a malaria species closely related to *P. vivax* that was previously considered to be a monkey-specific malaria parasite.

Human malaria parasites have a restricted host-specificity. They don't develop disease in rabbits, rats or mice but need to be maintained either in human volunteers or in primates. A common used less-than-optimal substitute is to perform experiments on primate, rodent or avian malaria parasites in their natural host. Most animal models are inadequate and, while they can help the researcher in answering specific questions, any extrapolation to human disease has to be considered with extreme caution. For example dexamethasone was considered to be useful in severe malaria caused by P. knowlesi in rhesus monkeys, but was found to be harmful in humans.

## Historical note

### Discovery of the parasite

Malaria has been with humanity since millennia. The most famous historical case of falciparum malaria is probably King Tutankhamen, the boy pharaoh from Old Egypt, in whose 3,000-year-old mummy the parasite was demonstrated. Although usually associated with the tropics malaria was endemic in North America and large parts of Europe until the middle of the 20th century. Malaria transmission occurred in Belgium, the Netherlands, Sweden, Finland and the United Kingdom. It was a significant impediment for the European nations during the colonial period. In Northern Europe, only *P. vivax* and *P. malariae* occurred. In Southern Europe, malaria was due to infection with *P. falciparum, P. vivax* and *P. malariae*.

In 1880 the French army doctor Charles Louis Alphonse Laveran discovered malaria parasites in fresh blood from malaria patients in the coastal town of Bone (Annaba), Algeria.

**Transmission**

The transmission of malaria had for long been a mystery. One of the researchers was the Briton Sir Ronald Ross. He left for India with a personal mission to prove transmission via insects. In 1897, after three years of hard work, he demonstrated parasites in mosquitoes which had bitten patients. Later he also demonstrated transmission of avian malaria via mosquitoes. He was able to describe the complete development of the parasite in the mosquito and also demonstrated that transmission took place via the bite of the mosquito (and not via the presence of dead mosquitoes in drinking water, as his mentor Patrick Manson had initially thought).

**Life Cycle**

After the cause and transmission of malaria became known, it was logical to assume that the parasites inoculated via a mosquito bite would directly penetrate red blood cells. This wrong idea was proposed in 1903 by Fritz Schaudinn, a distinguished German microscopist. It was based on faulty observation and due to his authority, it entered some textbooks. It was known that when blood from a patient with active malaria was inoculated into a healthy volunteer, the volunteer would develop malaria and would become infectious nearly instantaneously. However, when a volunteer was inoculated via a mosquito bite, the blood was not infective for 6 days (in case of *P. falciparum*) to 9 days (*P. vivax*).

Why? This was a vexing problem which took decades to answer. It was by very careful animal experiments with *P. cynomolgi*, a primate malaria species, that the puzzle was solved. *Shortt and Garnham* collected a large number of infected mosquitoes, mashed them to pulp and injected the lot (including sporozoites) into monkeys. After waiting a period, they killed the animals and searched the various organs and tissues. The parasites (with a different shape) were found in the liver. They had to support their hypothesis of the existence of a pre-erythrocyte stage with a species of human malaria. They used *P. vivax* and a human volunteer. This man was inoculated IV with sporozoites isolated from 200 mosquito salivary glands. A week later, the volunteer was operated on and a piece of liver tissue was obtained. The parasites were present in the liver. A year later they obtained a strain of *P. falciparum*, infected 770 mosquitoes and inoculated another human volunteer. About 6 days later a liver biopsy was taken and again the parasite was found.

**Pyrotherapy**

In 1927 Julius Wagner-Jauregg won the Nobel prize for his discovery of malaria pyrotherapy for treatment of late stage neurosyphilis. To induce repeated spikes of high fever in patients with progressive paralysis, he inoculated them with blood from patients who were suffering from tertian malaria (*Plasmodium vivax*). Although not without risk, this treatment proved to be very successful.

# Life cycle

When a mosquito lands on the skin, it attempts to pierce a small blood vessel with its proboscis in order to suck blood. To prevent the blood from coagulating the mosquito first injects some saliva. Besides vasodilating agents this saliva contains anticoagulants. However, the saliva may also contain micro-organisms. When a human is bitten by an Anopheles infected with malaria, parasites (sporozoites) [Gr. sporos = seed] are introduced into the human body. On average 10-20 sporozoites are injected per bite, although this number can be higher, e.g. 100.

A certain protein of the parasite, (the circumsporozoite protein, CSP), plays an important role in the penetration of the sporozoite into a liver cell (cf. Mosquirix vaccine). Sporozoites reproduce asexually in liver cells, by schizogony [Gr. schizo = split, divided]. This is called exo-erythrocytic or pre-erythrocytic reproduction. The form of the parasite produced in this way is called a liver schizont. The multinuclear schizont splits into many thousands of small offspring (merozoites) [Gr. meros = part]. Every successful sporozoite can produce some 20,000 merozoites.

After some time the infected liver cells burst and the merozoites enter the blood stream. While the parasites are reproducing in the liver, there are no symptoms. Neither the sporozoites, nor the liver forms are sensitive to most of the drugs used in malaria prophylaxis (atovaquone/proguanil is an exception). The minimal required time from infection to the appearance of the first merozoites, is the prepatent period. The incubation period is somewhat long because signs and symptoms do not appear until the parasitaemia is sufficiently advanced. Of note, merozoites in blood are usually too small to be seen by microscopy.

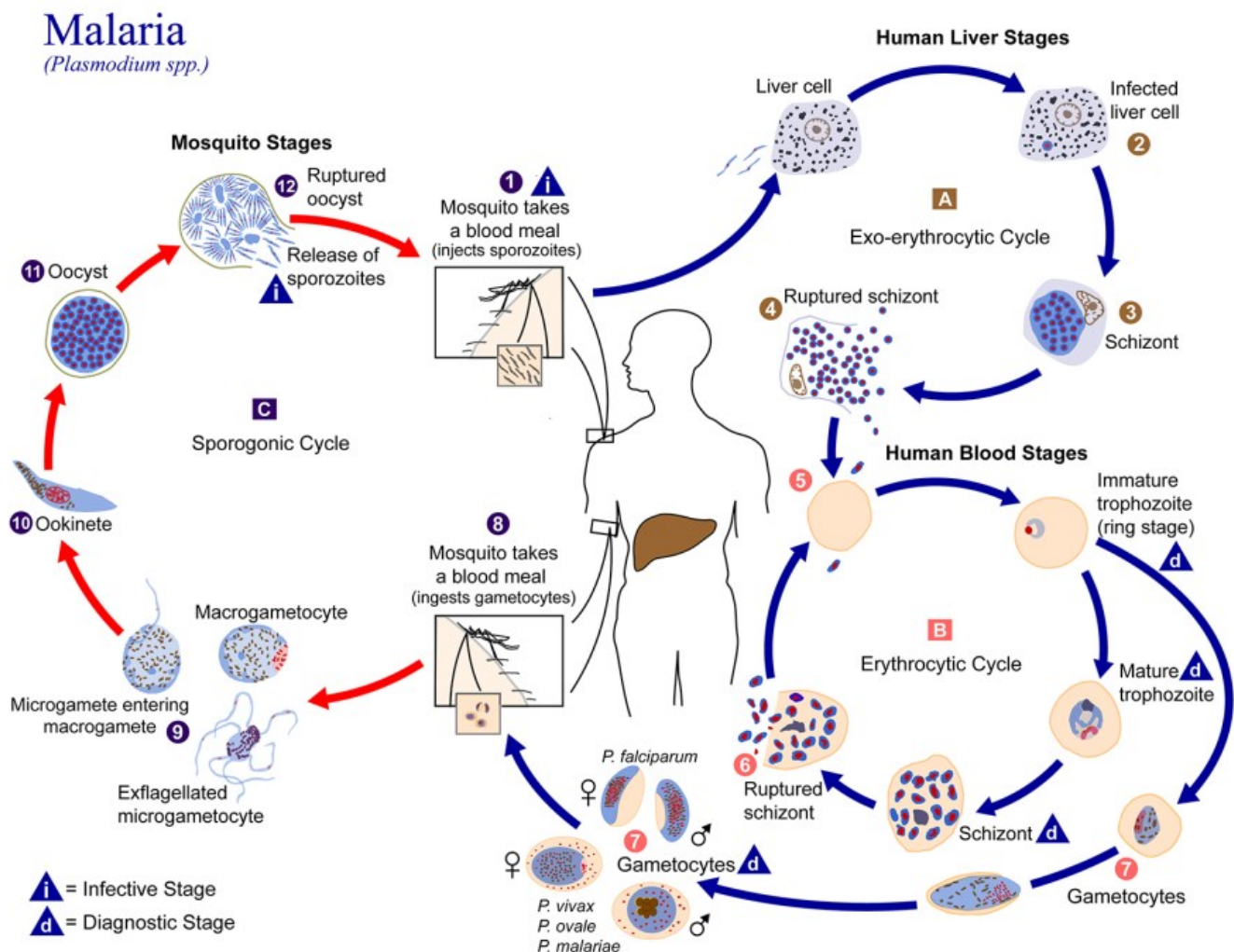In the case of *P. vivax* and *P. ovale* only some of the infected liver cells burst. The parasites in the liver cells which do not burst (hypnozoites) [Gr. hypnos = sleep] may remain viable for years and are responsible for new attacks of the disease if reactivated. The trigger which reactivates the hypnozoites is not known. The existence of hypnozoites in *P. vivax* was only formally demonstrated in

1985 via fluorescence microscopy. Reactivation of these "sleeping" forms explains delayed exacerbations of the disease after treatment with chloroquine and other antimalarial drugs. They kill the blood forms, but not the liver forms. Hypnozoites are not present in *P. falciparum* and probably not in *P. malariae* (although this is controversial). This is important for treatment because hypnozoites are not sensitive to chloroquine, quinine, mefloquine or artemisinin. Accidental inoculation with infected blood (blood containing trophozoites) may lead to infection, e.g. transfusion malaria or malaria via shared contaminated syringes by drug users. Since the infection in these cases is not transmitted by sporozoites, there are no liver forms. Liver forms are also absent in congenital malaria. This is important for treatment (no primaquine for congenital malaria with *P. vivax* or *P. ovale*). The chronic nature of infections with *P. malariae* is traditionally explained by assuming that the parasite can induce a very low parasitaemia (or hidden erythocytic schizontes) for many years, which is below the detection threshold of normal diagnostic methods.

In the red blood cell the parasite feeds on haemoglobin. The form of the parasite when present in the red blood cell is now known as a trophozoite (Gr. trophe = nutrition). The young parasite possesses a digestive vacuole with lysosomal enzymes. This vacuole contains proteinases (plasmepsin and falcipain). The vacuole can be clearly seen in a blood smear and explains the ring shape of the young parasite. The breakdown of haemoglobin results in an iron-containing pigment: hemozoin. The vacuole disappears as the parasite becomes older. The trophozoites will once more reproduce asexually and lead to the formation of a multinuclear parasite (schizont). The latter divides to form merozoites. Each schizont produces 8 to 24 merozoites, depending on the species, within a time span of 48 hours (*P. falciparum*, *P. vivax*, *P. ovale*), 72 hours (*P. malariae*) or 24 hours (*P. knowlesi*). The infected red blood cells burst after a while so that once more merozoites appear in the blood from where they will penetrate new erythrocytes within a few seconds. This bursting (lysis) of the red blood cells is accompanied by a bout of fever. If the development is synchronous (all parasites being at the same stage of development) the fever will follow a typical pattern (see below). This is, however, unusual: asynchrony is more common than synchrony, especially early in infections. The development from merozoite to schizont takes place in the peripheral blood and all stages can be observed. In *P. falciparum* usually only very young forms (ring forms) can be observed in the peripheral blood because older parasites (and schizonts) adhere to the endothelium of blood vessels in deep organs (e.g. the brain).

After a few days some of the merozoites transform into male or female gametocytes. These are necessary for sexual reproduction of the parasite (which only occurs in the mosquito). Gametocytes are responsible for transmitting the disease but do not themselves cause symptoms. Adult *P. falciparum* gametocytes are not sensitive to chloroquine and quinine, while those of *P. vivax*, *P. ovale* and *P. malariae* are sensitive. This means that following adequate treatment of *P. falciparum* there

may still be gametocytes in the blood, and this may continue for several weeks. This does not mean that the treatment has failed. One interesting hypothesis is that chloroquine might significantly increase the gametocytemia of chloroquine-resistant *P. falciparum,* resulting in an increased infectivity for *Anopheles*. This could, therefore, contribute to the rapid spread of chloroquine resistance.



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### Glucose metabolism and LDH

The trophozoite has no carbohydrate reserves and needs to consume glucose continually. The glucose metabolism in infected red blood cells is 50-100 times higher than that in non-infected cells. This

probably contributes to the hypoglycaemia which is often seen in severe infections.

The parasite have mitochondria, but these play a minor role in the provision of energy (the last word on this has not yet been said). Glucose is converted by anaerobic glycolysis to pyruvate and then to lactate. This latter step, as in humans, is catalyzed by the enzyme lactate dehydrogenase (LDH). The parasite's LDH is clearly different from that of humans and forms the basis of a diagnostic test (see below).

# Geographical distribution

Many lay people regard malaria as a purely tropical disease. However, the distribution of malaria used to be world-wide. Today, it still occurs in some 100 countries. The situation varies from region to region. Until 1938 there was still *P. vivax* malaria ("polderkoorts") in Belgium, and in the Netherlands as late as 1958, although there was an unexplained (possibly autochthonous) case of *P. malariae* infection in a child in Zeeland in 1969. The WHO declared the Netherlands officially malaria-free only in 1970. It is chiefly the pollution of surface waters which makes reproduction of *Anopheles* mosquitoes difficult.

Yet some *Anopheles* persist and can transmit malaria. *Anopheles atroparvus* is able to transmit *Plasmodium vivax* malaria but cannot transmit *Plasmodium falciparum*. *Anopheles plumbeus* can transmit tropical falciparum malaria. In the last century there were important changes in the lifestyle of humans, resulting in less human/mosquito contact. Effective therapy was available. All these factors mean that malaria has disappeared in Northwest Europe. Cases in Western countries are generally dealt with swiftly and satisfactorily and one person with malaria very rarely leads to the infection of others. Chronic large scale reintroduction of the disease in Europe is thus improbable, although with the combination of the current economic crisis with its plummeting health budgets, the massive influx of tropical migrants refugees and global climate change, makes this possibility more real at present than in the last decades of the 20th Century. To maintain an infectious disease, it is necessary for one infectious case to lead to one other infectious case, otherwise the disease will die out in the area. One would need sufficient gametocyte carriers and vectors to ensure the continuation of the disease.

Malaria is a very important public health problem in most tropical countries although the incidence rate of malaria declined globally between 2010 and 2018 from 71 to 57 cases per 1000 population at risk. In 2018, an estimated 228 million cases of malaria occurred worldwide, compared with 251 million cases in 2010. In that same year 405.000 people died of malaria mainly young children in Africa. Most lethal infections are due to Plasmodium falciparum. Six countries cause more than half of

all malaria cases worldwide: Nigeria (25%), DRC (12%), Ivory Coast, Mozambique and Niger (4% each). Of note, the decrease in incidence seems to stagnate these very last few years in sub-Saharan Africa.

P. falciparum is the most common form in sub-Saharan Africa (99,7% of malaria cases in this region),, tropical South America and Southeast Asia. The parasite occurred previously in the Mediterranean basin.

P. vivax has the widest distribution area (previously as far as London, Norway, Denmark, New York, southern Canada and even Siberia). In 1922 the number of cases in Texas was estimated at 500,000. It is the most common form in certain regions (e.g. Maghreb, Middle East countries, parts of China, Argentina). P. vivax preferentially penetrates young red blood cells (reticulocytes). In 1976 Miller discovered that P. vivax uses the Duffy blood group antigens (Fya and Fyb) as receptors to penetrate red blood cells. People who do not have this protein on their red blood cells cannot be infected with P. vivax. These antigens do not occur in the majority of humans in West Africa [phenotype Fy (a-b-)]. As a result, P. vivax does not occur in West Africa, or occurs in low numbers (and could be systematically missed). Duffy blood group negative erythrocytes are, in vitro, also resistant to infection with P. knowlesi (monkey malaria).

P. ovale is found chiefly West Africa, less elsewhere in Africa and sporadically in the Far East.

P. malariae is not very common but can be found anywhere. Often confused with P. knowlesi.

P. knowlesi is known from Malaysia (including Borneo), The Philippines, Vietnam, Thailand and recently Myanmar. The vector is present in India (Kerala) and Sri Lanka, but in these areas there is no known zoonotic reservoir. *P. knowlesi* infections are often confused with P. malariae.

Malaria can only persist naturally when climatic conditions are suitable for the vector(s) and for the development of the parasites in the vector. Increased rainfall and higher temperatures may make larger areas favourable for malaria transmission in the future. Tropical P. falciparum requires a minimum temperature of 18°C, while tropical P. vivax strains require a minimum of 16°C. The European strain of P. vivax which persisted in the high North was uniquely adapted, with summer temperatures being sufficiently high for P. vivax development in the mosquito. Infection of patients occurred in autumn (September / October) when mosquitoes started to enter homes looking for shelter. The P. vivax parasites in humans had a very long incubation period of 6 to 9 months. A patient infected with these northern strains of P. vivax would remain asymptomatic during winter until the following spring. This clearly differs from tropical P. vivax dynamics. In Southern Europe P.

falciparum used to be common in Portugal, Spain, Italy and Greece. It is likely that the strain of this parasite was genetically different from tropical strains.

However for malaria to become re-established, a sizable parasite reservoir (gametocyt carriers) must be present. This has not happened in other circumstances, such as after World War II, when great numbers of people (patients and gametocyt carriers) returned from tropical areas. In the current health system and socioeconomic situation in Europe, it is likely that patients will be treated early, diminishing the reservoir and lessening the threat of new epidemics. Small outbreaks and some local transmission might occur from time to time, but large reinvasion of the North European landmass is unlikely. South Europe would have a somewhat larger risk, as reflected by the outbreak of autochthonous *P. vivax* cases in Greece in 2012.

## Epidemiological classification – stable versus unstable malaria

There is no completely satisfactory epidemiological classification of malaria. Stable malaria means that the clinical disease is characterized by preferentially affecting children and achieving a protective "immunity" in adults. Stability does not mean that there can be no variation in transmission. In some regions seasonal malaria occurs. In other areas there is unstable malaria: transmission differs greatly from year to year and occasionally epidemics occur. Then the disease also occurs in older persons. This is important in many respects; as irregular control of malaria may lead to changes in the immune status of the population. Sometimes malaria may appear again in a region after a long absence. For example: in 1972 the disease was eradicated in South Korea following an intensive eradication campaign with case detection and vector control. In 1993 one case of P. vivax was observed. There then followed 22 cases in 1994, in 1995 there were 107 cases, 356 in 1996 and more than 1600 in 1997. In 1995 all cases were still limited to the border area with North Korea, but in 1996 there was also transmission outside the demilitarized zone. After entomological surveys had shown that *Anopheles sinensis* was the chief vector, measures were taken to control the disease.

## Vector, *Anopheles* mosquitoes

Malaria is transmitted via the bite of infected female *Anopheles mosquitoes.*

Malaria is transmitted by *Anopheles* mosquitoes. This applies to the malaria of all mammals. Avian malaria is chiefly transmitted by Culicinae. There are some 400 *Anopheles* species, 40 of which are good vectors while 28 are poor vectors. *Anopheles* mosquitoes are active at night. They do not buzz much and are not easily noticed. The world's most important vector is *Anopheles gambiae,* an anthropophagic and endophilic freshwater mosquito which flourishes preferentially in moist regions. It typically breeds in exposed sunlit and often transient aquatic habitats such as pools, puddles, and irrigation channels. *Anopheles* mosquitoes are good flyers: they can cover several kilometres in one night. This is of course of great importance for their control. Endophagic (bite inside the house) mosquitoes will often rest on walls after a blood meal. Residual insecticides which are applied there will kill the vector.

### How do mosquitoes find their prey?

Mosquitoes are attracted by an increasing $CO_2$ gradient. The warmth of the skin, lactic acid and moisture (breath) play a part over short distances. Every animal produces several volatile substances in its skin, breath, faeces and urine. A number of the substances (kairomones) are used

by the mosquito to find its prey. The details are complex. *Anopheles gambiae* prefers to land on the feet, while *A. atroparvus* prefers to bite the face.

### Vector control

Malaria vector control is primarily based on the use of insecticides. Appropriate monitoring of vector resistance to insecticides is an integral component of planning and evaluation of insecticide uses in malaria control programmes. Pyrethroids and DDT, two important insecticides used for vector control, block the nerve-impulse conduction by preventing a sodium channel from closing after an action potential. An important mechanism that confers resistance to pyrethroids and DDT, known as knockdown resistance or kdr, was first described in the housefly *Musca domestica*. It has been reported that a single mutation in the sodium channel sequence is the molecular basis of kdr in *Musca domestica*. The gene has also been characterized for *Anopheles gambiae*. PCR tests have been developed for the detection of the kdr-mutation in *A. gambiae*.

# Physiopathology

The incubation period may be short (minimum 7-9 days for *P. falciparum*) to very long (several years for *P. ovale*). In falciparum malaria the parasitaemia can be very high: up to 80% of erythrocytes may contain parasites, but even 5% is enough to result in severe disease. These situations may be life-threatening. The other malaria parasites produce much lower parasitaemia (especially *P. ovale*). They do cause severe illness but are rarely life-threatening. *P. knowlesi* infections mimics severe P. malariae infections.

The rupture of the red blood cells (haemolysis) is accompanied by fever, muscle pain and general malaise. Massive haemolysis may cause kidney failure. Parasitized red blood cells are removed by the spleen. Splenomegaly will result. Anaemia occurs due to the destruction of erythrocytes, suppression of the bone marrow and excess activity of the enlarged spleen (hypersplenism). In severe falciparum malaria, there is activation of blood coagulation system along with thrombocytopenia, even before widespread DIC and coagulation failure occur. In falciparum malaria there will often be a drop in glycaemia that can be corrected by administration of glucose.

The details of how cerebral malaria happens, are not clear at present, and various researchers have different opinions. More than 100 years ago, the Italian pathologists Bignami and Marchiafava reported on the sequestration of parasitized red blood cells in the brains of people who died of cerebral malaria. Erythrocytes which contain schizonts of *P. falciparum*, develop small knobs on their

cell membranes. These consist, among other things, of a histidine-rich protein, *P. falciparum* erythrocyte membrane protein 1 and rifins. Rifins are clonally variant proteins encoded by rif genes ("repetitive interspersed family") and are expressed at late ring or early trophozoite stage on the infected red cell surface. Their high copy number, sequence variability, and red cell surface location indicate an important role in host-parasite interaction. The knobs have an overall negative charge, allowing non-specific attraction to positive endothelial ligands, but specific molecular adhesion also play a part. With these knobs the infected cells cling to the walls of the capillaries and to the vascular endothelium of the post-capillary venules in the brain. The low local O2 pressure and high CO2 pressure are optimal for further maturation of the parasite. Infected red blood cells are less easily distorted and more rigid than normal erythrocytes. This impedes the blood flow, which can lead to cerebral malaria. Other organs may also be affected for example the placenta and the intestines (resulting in abdominal pain and diarrhoea). Red blood cells which contain schizonts of *P. malariae*, also develop knobs on their membranes, but these cells do not adhere to the vascular endothelium. When post mortem cerebral sequestration was compared with the peripheral parasitaemia, there were about 26 times more infected red blood cells in the brain microvasculature than in the peripheral blood if there were free-mixing. More blood vessels in the cortex and cerebellum than in the brain stem are affected. Some researchers found more sequestration in white matter than in cortex. Coma requires sequestration, but sequestration itself is not enough to provoke cerebral malaria. The rapid reversible nature of cerebral malaria led to the hypothesis that soluble neuroactive mediators might play a role in the pathogenesis possibly involving reversible disturbances of the blood brain barrier and biochemical disruption of normal metabolism.

There are two groups of parasites in *P. falciparum* infections: (1) the young forms in the peripheral blood which can easily be observed in a thin blood smear and (2) the mature group which is attached to small blood vessels and which cannot be seen. Falciparum schizonts are rarely found in peripheral blood but these are important for the development of cerebral malaria. The whole mechanism of cerebral malaria has not to date been fully explained. As well as the attachment of parasitized red blood cells to the vessel walls (cytoadherence) other mechanisms possibly also play a part. Normal red blood cells sometimes attach to parasitized cells, which impairs the microcirculation. All kinds of released chemical substances (cytokines, oxygen radicals, etc.) may also play a part. Cytokines such as tumour necrosis factor (TNF-a) increase the expression of receptor molecules on the endothelium and will contribute to the cytoadherence and flow obstruction which characterize falciparum malaria. This mechanism is similar to the release of TNF-a by endotoxins in Gram-negative septicaemia.

Increased brain volume was seen in children who died from cerebral malaria but was uncommon in those who did not die from the disease; this suggests that raised intracranial pressure may contribute to a fatal outcome.

Carriers of the sickle cell anaemia gene (heterozygotes for haemoglobin S) have relative protection against severe infection with *P. falciparum* and thus have a survival advantage (in homozygous patients, malaria may be fatal and the disease itself tends to kill patients before the reproductive age). The same advantage probably applies to persons deficient in G6PD. This may explain why these two conditions are so common in Africa. In Papua New Guinea ovalocytosis is common. These red blood cells have an oval shape and cannot be penetrated by *P. falciparum* parasites. Heterozygotes are thus protected against *P. falciparum* (homozygosity is not compatible with life).

In West Africa, haemoglobin C is rather frequent. People with haemoglobin AC or CC can be infected with *Plasmodium falciparum* and can develop substantial parasitaemia. The presence of Hb C therefore does not protect against infection itself. Haemoglobin C might protect against the lethal effects of *P. falciparum* malaria by reducing cytoadherence of parasitized erythrocytes.

Haemoglobin E (chiefly Southeast Asia) does not protect against *P. falciparum* infections itself.

While circulating in human blood *P. falciparum* exhibits antigenic variation. On the surface of the infected red blood cell a certain protein is expressed: *the P. falciparum* erythrocyte membrane protein 1 (PfEMP-1). The parasite can make many variants of this protein. By interchanging which variant of PfEMP-1 is present, the parasite can evade the immune response to these immune dominant antigens. These proteins are thought to be the major virulence factor found on the surface of infected red blood cells, directly contributing to the pathogenic nature of the infection and placing these genes at the centre of a disease responsible for several million deaths in developing countries. Although there are many var gene copies, only a single var gene is expressed at any given moment (i.e. there is mutually exclusive expression). Over the course of an infection, expression switches from one var gene to another, resulting in antigenic variation of the parasite population and a persistent infection which is difficult to clear by the human immune system.

Antigenic variation has important implications for the development of vaccines. The repertoire of proteins which are expressed in the *Anopheles* mosquito is far less pronounced probably because the vector has no adaptive immune system.

A large case-control study of malaria in West African children showed that a human leukocyte class I antigen (HLA-Bw53) and an HLA class II haplotype (DRB1*1302-DQB1*0501), common in West Africans but rare in other racial groups, are independently associated with protection from severe malaria. In this population they account for as great a reduction in disease incidence as sickle-cell trait. These data support the hypothesis that the extraordinary polymorphism of major histocompatibility complex genes as well as other genes has evolved primarily through natural

selection by infectious pathogens.

Malaria is very often accompanied by thrombocytopenia, the causes of which seem to be multiple and not completely known. The severity of the thrombocytopenia correlates with the parasitaemia and the clinical severity of infection.

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# Malaria – Clinical aspects

## Classic acute uncomplicated attack

Most clinical episodes of malaria are characterized by fever with aspecific symptoms. Certainly, in children the presentation can be very misleading. Any fever should bring the possibility of malaria to mind. There is a danger, however, that every fever episode may be regarded as malaria and other important diagnoses are then likely to be missed.

*P. falciparum*: typical incubation time: 7 to 30 days. If a person is taking preventive antimalarials and if the parasite is partially resistant, there may be temporary suppression of a malaria attack. The fever is generally irregular. If the attack is not treated, after a few weeks a regular fever pattern will develop with peaks every 2 days (tertian malaria, so called because the fever reappears on the third day, reckoning the day of the paroxysm as the first. This is rare in everyday clinical practice). At the beginning of the attack the symptoms are similar to influenza: general malaise, tiredness, muscle pain, headache but in general without respiratory tract problems or running nose. These symptoms are non- specific. After a while the muscle pain and headache become worse. Sometimes there is also abdominal pain and diarrhoea. Rarely there is a classic attack: this lasts for approximately 12 hours and occurs every 48 hours. At first cold shivers with high fever occur, followed by an intense feeling of heat and fever, leading to a sweating stage with a drop in fever. Most falciparum attacks do not follow this classic pattern. Therefore what is referred to as a classic attack is paradoxically not the general rule.

*P. vivax* and P. *ovale*: the incubation time is a few weeks to years. The awakening of dormant parasites in the liver (hypnozoites) explains the potential for late relapses. The fever is sometimes regular (every 48 hours) especially in cases of recrudescence (tertian malaria). In 1922 *P. vivax* was

introduced for the treatment of neurosyphilis. It was thought that the bacterium which causes syphilis had little resistance to heat, so the high fever would kill the bacteria (*Treponema pallidum*).

*P. malariae*: the incubation time is 3 weeks to many years. The very late attacks are probably not due to awakened hypnozoites (to date these have never been detected) but due to the activation of blood parasites which are present at a very low concentration. Fever peaks may occur every 72 hours (quartan malaria).

*P. knowlesi* is a monkey parasite which can be misidentified as *P. falciparum* in the early ring stage and as *P. malariae* in the older stages. It has the shortest asexual life cycle of all i.e. 24h. The prepatent period is 9-12 days. At present, no hypnozoites have been found. PCR is needed to firmly identify this species.

Mixed infections: mixed infections do occur, but for reasons which are unclear they are much less common than would be expected based on the prevalence of the individual species. Underreporting may play a part, but this is probably a real phenomenon (partial cross-immunity to heterologous species? biological interference?).

## Natural course of malaria in the autochthonous population

Children are very susceptible to infection. The highest mortality is found in children below the age of 5 years. Gradually, after repeated infections, a partial immunity develops in those who survive. There is a high degree of tolerance to the infection in adults, provided they live in a stable malaria region. This semi-immunity (or "premunition") is maintained by repeated infections and mild latent infections. It disappears after approximately 6 to 24 months if there is no further infection (e.g. a stay in a non-malaria region).

This partial immunity is reduced during pregnancy. A pregnant woman is at increased risk of hypoglycaemia and cerebral malaria. Malaria is an important cause of severe (sometimes spectacular) anaemia in the mother, low birth weight, premature birth, abortion and increased perinatal death. Chondroitin sulphate and hyaluronic acid, both present in abundance around the syncytiotrophoblasts of the placenta, are mucopolysaccharides (glycosamine glycanes) which act as receptors for red blood cells infected with *P. falciparum*. Probably there are also other receptor molecules. Infected cells accumulate in the placenta resulting in reduced placental function.

The placental barrier is very seldom passed. Congenital malaria is not common and occurs chiefly in

neonates of non-immune women. Neonates of semi-immune women receive transplacental anti-plasmodium antibodies. Due to this passive resistance in the first 3-6 months they are at a lower risk of malaria.

Several observations of humans infected with both malaria and helminths suggest that co-infection provides a benefit to either parasite. The evidence indicates that malaria patients co-infected with helminths are protected from severe malaria possibly through skewering of the immune response towards T helper (Th)2 immunity.

Malaria and HIV interact in several, rather complex ways. The CD4+ lymphocytes play a central role in the defence against malaria and their characteristic decrease during the course of HIV infection explains why severely immunosuppressed HIV-positive individuals are so susceptible to severe malaria. In malaria-endemic regions, severe malaria may be considered as an "opportunistic infection" and any diagnosis of complicated malaria in adults should trigger HIV testing.

# Acute severe malaria

Acute severe falciparum malaria is a medical emergency. This encompasses:

- Coma: repeated generalized convulsions
- Hypoglycaemia: reduced consciousness, aggressive behaviour
- Severe anaemia: weakness, tachypnoea, pale mucosae
- Tendency to spontaneous bleeding (pronounced thrombocytopenia)
- Circulatory collapse (shock); cfr. below "algid malaria"
- Pulmonary oedema (dyspnoea and bilateral crackles) leading to acute respiratory distress syndrome (ARDS)
- Haemoglobinuria (dark urine)
- Kidney failure: urinary output should be monitored (but attempts to force urine production may cause circulatory overload!)
- Acidosis (chiefly due to lactic acid): tachypnoea. If too many salicylates are given this may exacerbate acidosis (not unusual in febrile patients).
- Other important signs are: marked jaundice, confusion without coma, extreme generalized weakness or prostration (child cannot remain sit and don't want to eat/drink).

The priorities are cerebral involvement, severe anaemia, hypoglycaemia, and the presence of hyperparasitaemia. The degree of parasitaemia correlates with the severity of the symptoms: the higher the parasitaemia, the greater the risk of severe symptoms. It should be borne in mind that the

parasitaemia (the percentage of parasitized cells that are found in a smear preparation) changes by the hour. This is because the red blood cells with mature *P. falciparum* parasites (schizonts) attach themselves to the small capillaries of deep organs therefore are not found in a thin blood smear. A parasitaemia of 0.5% is already severe, 2% is pronounced, and patients with a parasitaemia of more than 10% have a relatively poor prognosis. Over 25% is often fatal. Another consideration is that a parasitaemia of 3% in someone who still has a normal red blood cell count, is different from a parasitaemia of 3% in an anaemic patient.

**Hypoglycaemia** may quickly lead to general deterioration and coma. It is common in children (up to 25% of cases) and pregnant women. Glucose may be life-saving. If the glycogen store in the liver is low (i.e due to malnutrition) the risk of hypoglycaemia increases [glycogen is converted to glucose]. The conversion of glycogen to glucose is also inhibited by certain cytokines which are released during infection with *P. falciparum* [Hypoglycaemic effects of TNF-a and possibly interleukin-1 and TNF-ß]. The parasites themselves also use glucose for their metabolism and contribute to the hypoglycaemia if they are present in large numbers. Quinine can stimulate the secretion of insulin from the pancreas and in this way can also contribute to hypoglycaemia.

The term **"algid malaria"** (L. "algidus" = cold) is obsolete. The condition is characterized by hypotension with progression to shock. The patient is clammy and often feels cold. There is no fever. Often there is concurrent septicaemia with Gram-negative bacteria. Mortality is high. Therapy with artesunate or quinine, treatment with antibiotics and (cautious, see ARDS) IV fluid administration is of great importance. Shock seldom occurs in malaria if there is no septicaemia. However splenic rupture can also cause hypovolemic shock.

**Splenic rupture**. This may occur spontaneously or after an unobserved trauma. This complication can occur in *P. falciparum, P. vivax, P. ovale* or *P. malariae*. The presence of intraperitoneal fluid is suggestive in this context. In these cases ultrasound can often detect a splenic hematoma, splenic rupture or intraperitoneal fluid. A diagnostic peritoneal lavage may be indicated.

**Cerebral malaria** is the main cause of death (80 %) in falciparum malaria. This complication occurs chiefly in non-immune persons (children, travellers). Cerebral signs include confused behaviour, psychosis, convulsions, stupor, coma, paralysis. Unlike meningitis, there is no real neck stiffness (pain) or photophobia (intolerance to light) but neck retraction and opisthotonos (neck muscle spam) may occur. Sometimes the difference between neck stiffness and neck retraction is not clinically clear. It is typical of the coma that it develops swiftly in 75% of cases and quickly disappears. If a child survives cerebral malaria it has approximately a 10% chance of significant long term sequelae. Children with cerebral malaria and with a normal eye fundus have a good prognosis, while

papilledema and retinal bleeding suggest a guarded prognosis. Malarial retinopathy is increasingly considered as a specific diagnostic criteria of cerebral malaria, but sensitivity of this abnormality is rather poor (meaning that its absence does not exclude cerebral involvement). Repeated generalized convulsions should not be regarded as "normal" febrile convulsions. Severe convulsions with contraction of the abdominal muscles and compression of the stomach, may cause reflux of gastric acid and food into the pharynx. Aspiration of gastric contents into the lungs is a real danger as this may result in Mendelson's syndrome (chemical pneumonitis) or aspiration pneumonia. If there are convulsions, these are stopped by administering diazepam (Valium®) IV. A CT scan or MRI scan of the brain of patients with cerebral malaria shows few abnormalities except an occasional increase in cerebral volume. Herniation of the brain stem is a rare event.

If confronted by a febrile coma or confusion with fever in the tropics, glucose must be administered (preferably IV), artemisinin (or quinine if artemisinin unavailable) therapy should be instituted and a lumbar puncture carried out without hesitation (to rule out meningitis). Of the persons who die in hospital due to cerebral malaria, 50% of the fatalities occur within the first 12 hours after admission. At autopsy countless petechiae can be seen in the brain. Small ring-shaped haemorrhages also occur around cerebral blood vessels.
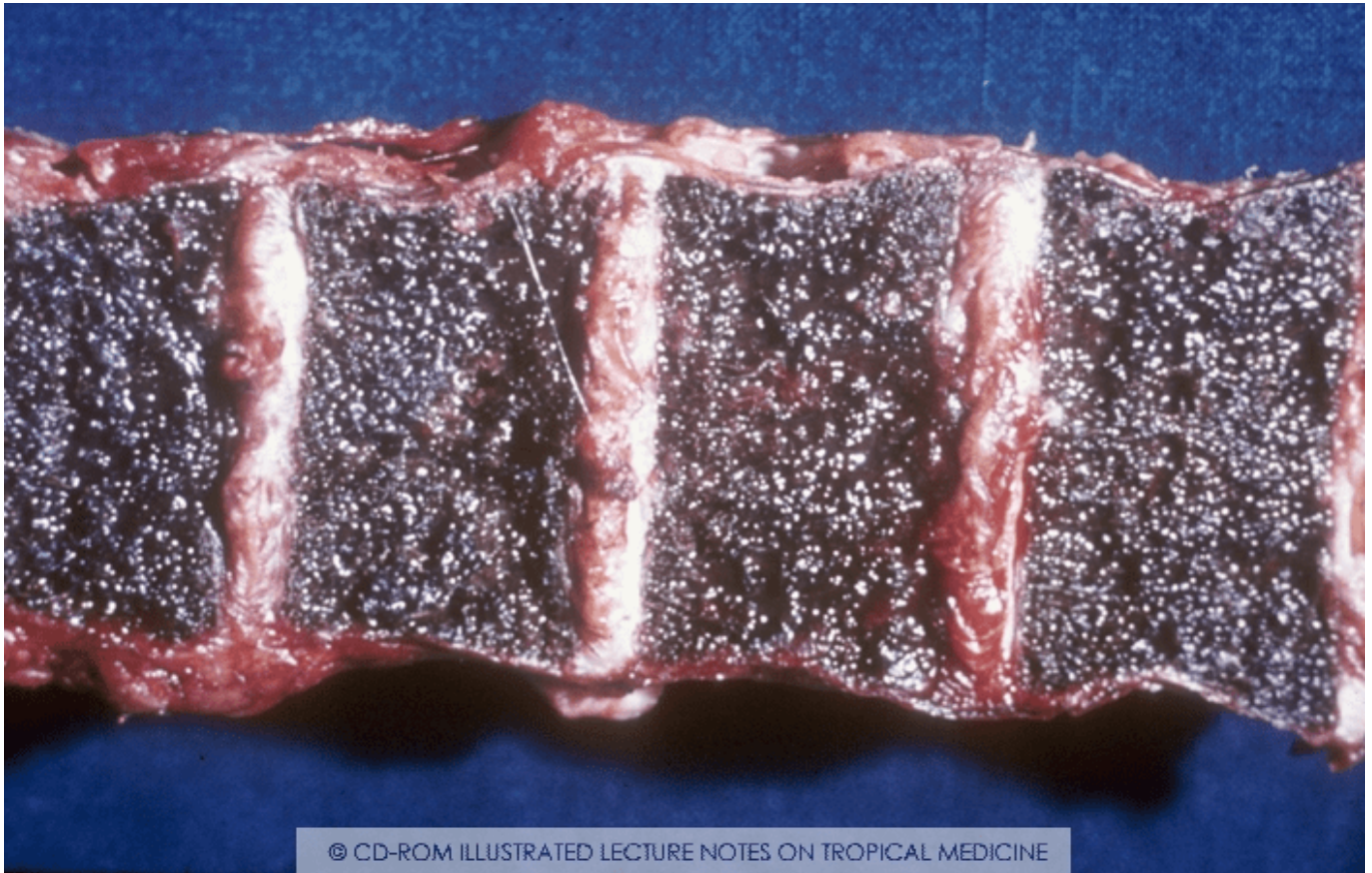
### Febrile convulsions

Febrile convulsions are generalized tonic-clonic convulsions. They only occur in children between the age of 6 months and 5 years and will not be repeated during the same fever episode. They occur during the phase in which the fever is rising fast. They always last less than 15 minutes, but post-ictal coma can take up to 1 hour. There is never postictal hemiparesis. It is important to differentiate between febrile convulsions and convulsions during fever (e.g. cerebral malaria, meningitis, cerebral abscess). Approximately 2% of children have a tendency (possibly genetic) for febrile convulsions. The risk that epilepsy will develop in this group of patients is no greater than in children without febrile convulsions. Brief and sporadic attacks have a good prognosis. No maintenance therapy with anti-epileptic agents is required.

**Severe anaemia** occurs due to haemolysis (of both parasitized and non-parasitized red blood cells – the latter via immune-mediated mechanisms), due to excessive action of the spleen i.e. hypersplenism (until weeks after the infection), due to possible haemorrhages (low blood platelets, splenic rupture) and due to disturbed production of new blood cells in the bone marrow (dyserythropoiesis) due to TNF-alpha.

**Hyperpyrexia** (very high fever above 40°C) should be treated by cooling the patient and administering paracetamol. It is assumed that malaria fever is caused when lysis of the red blood cells releases malaria pigment (hemozoin) as well as GPI-anchors ("malaria toxin") which are absorbed by the reticulo-endothelial system. This in turn releases endogenous pyrogens (cytokine network). The concentration of tumour necrosis factor in the peripheral blood correlates with the severity of the malaria. In cases of repeated malaria attacks the liver, spleen and bone marrow are stained black by the enormous amounts of hemozoin. Hyperpyrexia is no longer considered as a criteria of severity (WHO classification of 2000).

# Black water fever

Black water fever is a severe life-threatening complication. Acute massive haemolysis occurs through immuno-allergic mechanisms which are not fully understood. It has been observed after taking halofantrine, artemisinin-derivatives and after irregular use of quinine. The parasitaemia is generally very low. There is high fever, jaundice, back pain, shock and very dark urine. Renal insufficiency occurs: the urine production is very low (oliguria) or zero (anuria). Mortality is very high. When quinine was no longer used prophylactically, black water fever became very rare. Differential diagnosis should be made with severe malaria itself, leptospirosis and viral haemorrhagic fever.

Black pigmentation of the bone marrow in the spine, due to accumulation of malaria pigment (repeated malaria). Photo Dr Gigase. Copyright ITM

Acute renal failure may also be caused by shock, hypovolemia with reduced renal circulation, disseminated intravascular coagulation (DIC), obstruction of the renal glomeruli by parasitized red blood cells and by the precipitation of released haemoglobin in the kidney (pigment nephropathy). The combination of these factors can result in acute tubular necrosis. Glomerulonephritis may occur in chronic *P. malariae* malaria (cfr. infra), but this complication plays no part in acute renal problems.

**Pulmonary oedema** is a common complication of severe malaria. The dividing line between overhydration and dehydration is narrow. Adults easily develop non-cardiogenic pulmonary oedema if there is excessive fluid overload, but on the other hand dehydration and hypovolemia may lead to hypotension, shock and renal failure. Pneumonia is observed quite often if coma lasts for longer than 3 days. ARDS (acute respiratory distress syndrome) may occur. This is caused by diffuse damage to the vascular endothelium and the alveolar epithelium. There is a rapid progression towards dyspnoea, arterial hypoxia, bilateral patchy pulmonary infiltrates due to pulmonary oedema with a protein-rich

fluid. The treatment is both etiological and symptomatic: mechanical ventilation, with or without intubation or an endotracheal cannula, possibly with NO (nitrogen monoxide), high-dosed oxygen and positive end-expiratory pressure (PEEP).

# Chronic falciparum malaria

Where *P. falciparum* is partially resistant to the therapeutic drug locally used (e.g. chloroquine), the parasite may be suppressed, but will remain present (not completely cleared). This may lead to a whole range of clinical pictures, from asymptomatic parasitaemia through to mild unspecific symptoms, to significant chronic malaise, anaemia and fatigue. Curative therapy with atovaquone/proguanil or artemisinin-based combination therapy (ACT, see below), for example, produces rapid improvement.

# Hyperreactive malaria splenomegaly (HMS)

Some adults have a very strong immunological reaction to *P. falciparum* antigens. The level of IgM in the blood is very high. Due to the polyclonal immune stimulation, all kinds of autoantibodies can appear. Immune complexes are formed, and are removed by the reticulo-endothelial system, which leads to splenomegaly and sometimes hepatomegaly. In these individuals the swollen spleen swells also breaks down normal, non-parasitized red blood cells. The number of parasites is very low, but very high concentrations of anti-*P. falciparum* antibodies can be detected. The splenomegaly disappears after curative therapy with, e.g. ACT followed by months or even years of adequate malaria chemoprophylaxis if persistent exposure in a malaria region), but recovery is very slow. In rare cases splenectomy is necessary. Steroids have no place in the treatment.

> **HMS and splenic lymphoma**
>
> HMS may be very similar to a certain indolent splenic lymphoma (e.g. splenic lymphoma with villous lymphocytes). The latter disorder is related to B-cell chronic lymphocytic leukaemia and occurs chiefly in elderly persons. The disease is often accompanied by significant cytogenetic abnormalities and monoclonal "villous" B-lymphocytes in the peripheral blood. It is likely that in HMS, excessive stimulation of the B-lymphocytes by malaria antigens increases the risk that oncogenic mutation may occur, followed by clonal growth of these cells.

# Burkitt's lymphoma

This malignant tumour originates from B-lymphocytes. It is very aggressive with a volume doubling

time of about 3 days. The endemic form occurs in sub-Saharan Africa and is also found in Papua New Guinea. In these areas, it accounts for up to 50% of childhood tumours.

One hypothesis states that repeated malaria attacks may have a mitogenic effect on infected B-lymphocytes (polyclonal B-cell stimulation) increasing the risk of mistakes during chromosomal replication which subsequently would lead to neoplastic behaviour.

Burkitt's ymphoma generally presents with swelling of the jaw and mouth ulcerations (75%, especially maxilla tumours), abdominal swelling with ascites (60%) and central nervous system involvement (30%, including cranial nerve palsies, malignant pleocytosis or paraplegia). Infection with the Epstein-Barr virus (cf. mononucleosis) plays an important part in the endemic form of Burkitt's lymphoma, probably by causing genetic instability. Epstein-Barr viral DNA is found in about 90% of African Burkitt's lymphomas.

The tumour responds well to cytostatic drugs. The alkylating agent cyclophosphamide (Endoxan®) is first choice (the target dose 1-1.5 gram/m2 IV every 3-4 weeks with 2 doses in remission), but more complex chemotherapies (methotrexate, vincristine, CHOP-R, hyper-CVAD,...) are difficult to evaluate in low-resource settings.  About 80% of patients can achieve complete tumour regression and 10% have a partial response. About 50% will relapse.

Nephrotic syndrome secondary to chronic infection with Plasmodium malariae. Notice the swollen face and ascites. Photo prof. Gigase. Copyright ITM



Burkitt's lymphoma in a Cambodian woman, aspect before chemotherapy. Photo Dr Lut Lynen, copyright ITM

# Nephrotic syndrome in P. malariae

Chronic infection with *P. malariae* may, via immunological mechanisms (chronic immune complex glomerulonephritis) cause a nephrotic syndrome, characterized by oedema and proteinuria (more than 3.5 gram per 24 hours). There is often significant hyperlipidaemia and lipid bodies are sometimes found in the urine.

If a kidney biopsy is carried out, it should be borne in mind that severe bleeding will occur in 1% of cases. The treatment of nephrotic syndrome is difficult. Curative malaria treatment is of course

indicated but will not produce improvement of the kidney function. Salt restriction and diuretics are indicated (both thiazide and loop diuretics). Treatment with an ACE-inhibitor [angiotensin-converting enzyme-inhibitor such as enalapril] should be ideally initiated in settings where it is available. Steroids and immunosuppressive agents are of little benefit in this disorder. An important challenge is to distinguish the entity from minimal change glomerulonephritis (electron microscopy needed to confirm "minimal change" on biopsy specimen).

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# Malaria – Diagnosis

## General

When can one assert that someone has the disease "malaria"? There are several problems and the question has still not been fully resolved. The demonstration of malaria parasites in the blood is essential but insufficient in itself. Most cases are accompanied by thrombocytopenia and normal white count. Many people will develop an acquired immunity after several years of exposure and may harbour parasites without exhibiting symptoms. The degree of parasitaemia may help, but there is no absolute criterion (the higher the parasitaemia, the more chance that malaria is in fact the diagnosis). There are patients with malaria for whom the thick smear is negative (luckily this is rare in a good laboratory). There are no pathognomonic clinical signs. An accurate diagnosis is becoming more and more important, in view of the increasing resistance of *P. falciparum* and the higher price of modern combinationtreatments.

## Clinical aspects

No single clinical sign allows the diagnosis of malaria. Most cases are accompanied by thrombocytopenia, a normal white count and a positive parasitaemia. Yet malaria must always be considered in cases of fever in the tropics. Since the symptoms can be quite diverse, a clinical diagnosis is unreliable and the diagnosis should be based on identification of the parasite. Microscopic confirmation of the diagnosis is often not possible in many regions and situations. It is of the greatest importance that other important diagnoses are ruled out before instituting a blind anti-malaria therapy. All too often fever is considered as malaria without considering alternative diagnoses. This tendency is reflected in the quote: "if you only have a hammer, you tend to see every problem as a

nail" (Abraham Maslow).

The presence of parasites does not rule out an additional diagnosis: e.g. someone with fever may well have some malaria parasites in a thick smear, but this does not rule out meningitis or pyelonephritis. Chronic carriers are people who, although they have malaria parasites in their blood, have no symptoms of this. When such people develop another infection their symptoms are often attributed to the malaria parasites in their blood, although these are not responsible. The absence of parasites in a single preparation does not rule out malaria but does make the diagnosis of *P. falciparum* highly improbable (if the microscopist searched carefully). Where there is any clinical suspicion it is best to repeat the test 12h later.

## Microscopy

A **thick smear** concentrates the parasites 10 to 25 times. It is rather more difficult to interpret than a thin smear preparation and often does not permit species identification. A thick smear contains no intact red blood cells (haemolysis due to the distilled water used in the staining). If a thick smear is positive, a thin smear should be examined.

### Parasitaemia

The parasitic density can also be roughly determined in a thick smear, by counting the number of parasites per 200 leukocytes and multiplying this by 30. It is assumed that on average there are 6000 leukocytes per µl blood and that there is one leukocyte per 500 red blood cells. For example: 5 parasites per leukocyte (1000 parasites for every 200 leukocytes) corresponds to a density of 30,000 parasites per µl. Roughly 30,000 parasites per µl corresponds to a parasitaemia of 1% (5 parasites per 500 RBC's): a moderately anaemic person.

If the thick smear is found to be negative in a reliable laboratory and if there is strong suspicion of malaria, the test is repeated every 12 hours for 48 hours. One great disadvantage of the thick smear method is that reliable technical expertise is needed which should be monitored (e.g. quality control). The argument that a lab technician has carried out the test for years and thus has plenty of experience is absolutely no guarantee of quality or reliability. The test also requires plenty of time if the parasitaemia is low, or before a negative result can be concluded.

A **thin blood film** has many advantages:

- it demonstrates the species present

- detection of mixed infections is possible
- distinguish asexual stages from gametocytes
- assesses parasitaemia (in % of infected red blood cells)
- can detect a new or unexpected parasite
- gives information on red cell morphology
- allows a white cell differential count
- inexpensive

Other points include: Sensitivity and specificity is operator dependent. In a good average lab, the sensitivity is good but limited to about 50 parasites per µL, this is somewhat better in a reference lab. Most routine laboratories cannot detect parasitaemia below 100 to 500 parasites per µL. DNA amplification techniques have better sensitivity and can give information when species is in doubt but this technique remains limited to reference laboratories (even in high resource settings).

If the parasite cannot be identified it is regarded as a *P. falciparum* as a safety precaution. Mixed infections do occur.

# Antigen detection

Malaria rapid diagnostic tests (RDTs) based on lateral-flow immunochromatography are increasingly used in endemic and non-endemic settings. They are easy to use, provide results rapidly and require no specific training and equipment. Reported sensitivities vary between different RDT products but are generally good for *Plasmodium falciparum*, with rapid tests based on the recognition of *P. falciparum* antigen **histidine-rich protein-2** (PfHRP2) scoring slightly better than those which recognize *P. falciparum*–**lactate dehydrogenase** (LDH). Sensitivity is lower for *Plasmodium vivax* (66 – 88%) and usually poor for *Plasmodium ovale* (55 – 85%) and *Plasmodium malariae* (21 – 45%). Rapid diagnostic tests have some limitations. The test strips are susceptible to heat and humidity. A positive result can be obtained after correct treatment, when there are no more parasites visible in the thick blood smear. This is due to persistence of the PfHRP2 antigen (up to several weeks) after successful treatment. The pLDH based tests have the advantage of turning negative sooner after parasite clearance (several days). Occasionally there is cross-reactivity of *P. falciparum* with the non-falciparum test line and vice versa and rare false-positive reactions due to other infectious agents or immunological factors. False-negative results occur in the case of low parasite densities, prozone effect (saturation of binding sites due hyperparasitaemia) or pfhrp2 gene deletions as observed in Pf strains from South America, but also in Mali, DRC and India. The latter two reasons for false negativity are only observed with HRP2-based RDTs. Finally when instructions are not followed (delayed reading, incorrect sample and buffer volumes, not recognizing invalid test results, disregarding faint test lines)

errors in interpretation can occur. **Rapid diagnostic tests do not give information about parasite density.**

### Depolarized light scatter

Automated cell counters, such as certain Cell-Dyn instruments, use 90° depolarized light scatter to distinguish eosinophils from other leukocytes. Eosinophils are normally the only leukocytes that depolarize light. Some automated haematology analyzers display an alert for possible malaria based on the presence of  activated monocytes (Coulter Counter), hemozoin containing white blood cells (Cell-Dyn series) and an additional peak in the reticulocyte fraction (Cell-Dyn series). During malaria infection, the parasites consume haemoglobin and produce malariapigment, a form of polymerized haeme. This pigment, also known as hemozoin, is birefringent. When peripheral blood is analyzed by automated flow cytometry, the pigment will cause atypical depolarization of the laser beam that can be recognized in a scatterplot. Although diagnostic accuracy of these features is too low to exclusively rely on these flags for malaria diagnosis, such an alert is especially useful in situations where the initial clinical suspicion of malaria is low (non-endemic setting).

## PCR

At present, in case of doubt, mixed infections, low parasitaemia, forensic questions, suspicion of zoonotic malaria, etc… PCR technology (e.g. multiplex real-time PCR) can give answers to several questions, but is in general slower than the traditional methods since such tests are not performed everyday even in larger centres. However, point-of-care PCR based techniques are being developed and their importance might grow in the future in countries contemplating malaria elimination, especially if this technique can combine detection of multiple infectious agents (multiplex-PCR). The future will learn whether they will have a place in diagnosis even in low-resource settings.

## Serology

Serology can only be carried out in reference hospitals and is of no importance for the individual diagnosis in acute fever. The antibodies are positive from the tenth day therefore at the beginning of the attack they will be negative. The presence of antibodies only shows that there has been contact with the parasite. This does not mean that there is immunity. There will be high titers of antibodies in the tropical hyperreactive malaria splenomegaly. Malaria type IgG antibodies penetrate the placenta and will give the neonate temporary and partial protection against malaria during the first months of life. Antibodies after infection remain positive for a longer time.

## Indirect aspects

Signs of haemolysis include yellow serum, dark urine while faeces have a normal colour, elevated indirect bilirubinaemia and low haptoglobin. Often there is thrombocytopenia. Sometimes there is malaria pigment in white blood cells (sign of severity).

## Test therapy

In endemic regions fever, muscle pain or even generally feeling unwell are often attributed to "malaria". An anti-malaria treatment is then instituted, without obtaining confirmation of the diagnosis or often even without considering alternative diseases. The argument given is that such a treatment can do no harm, that the diagnosis of malaria is always probable because the disease is common and that this is a good strategy for first-line care. Each of these arguments can be defended to a certain extent, but in this way often useless and sometimes expensive treatments with potential side effects are administered. In addition, not recognizing and treating other diseases (borreliosis, rickettsiosis, kidney infections, amoebic liver abscess, pneumonia, sepsis and so on) is a daily reality in many tropical regions. The over-diagnosis of malaria often leads to under-diagnosis of other treatable disorders. It is sometimes stated that fever which does not disappear after three days of adequate therapy, is not malaria. This may however not be completely true, in case of drug-resistant malaria (resistance R3, with no decrease in the parasite load during treatment) or co-infection with another pathogen (commonly sepsis).

In face of the increasing resistance to *P. falciparum* parasite and the need of more complex and expensive treatment (ACT), WHO recommends since 2010 the diagnosis of malaria being parasite-based as often as possible either by microscopy or antigen-based RDTs. Ideally no malaria treatment should be provided without confirmation of the diagnosis.

LAST UPDATED BY ADMIN ON JANUARY 14TH, 2025

# Malaria – Treatment
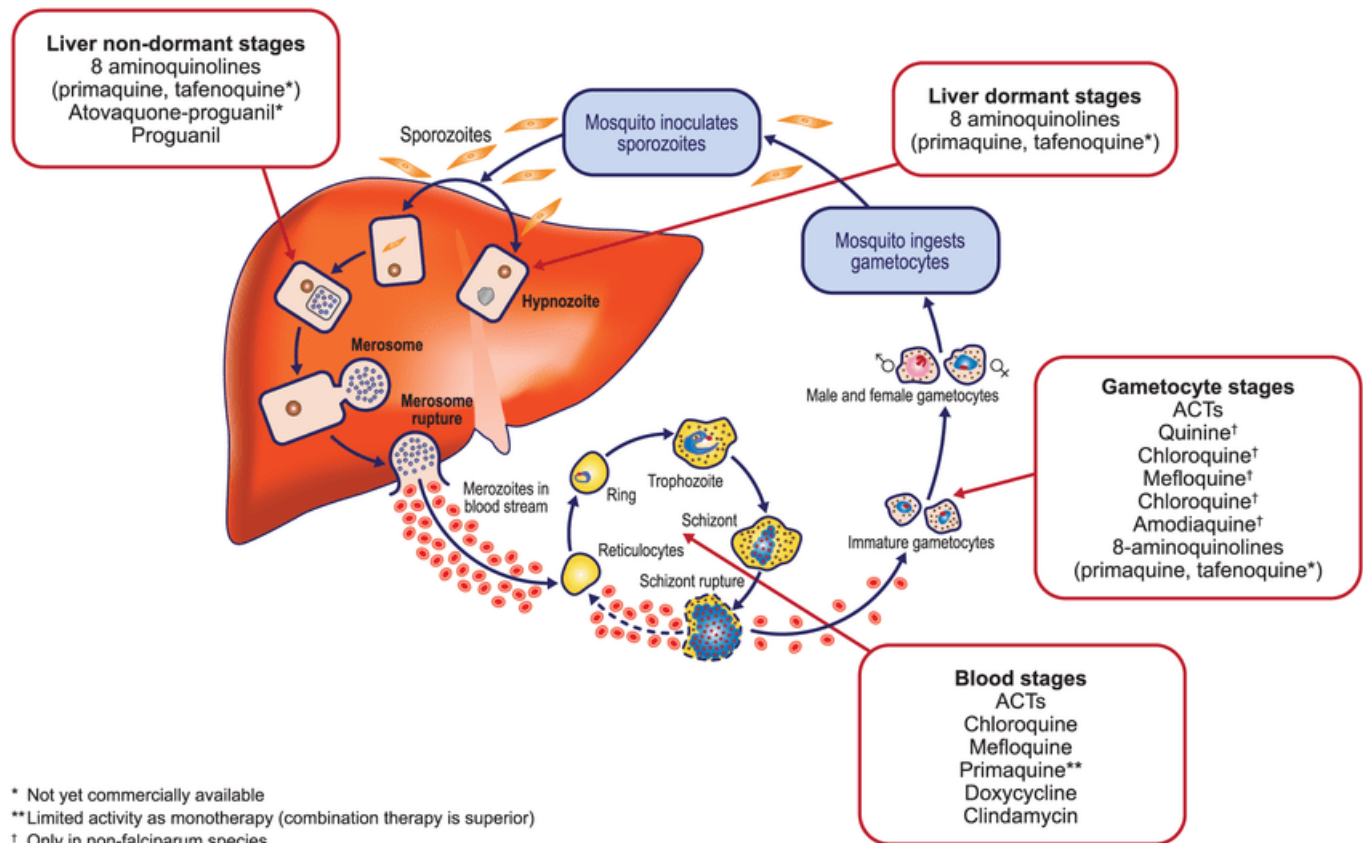
## Specific anti-malaria drugs

# General

Most people are not very interested in the history of a particular medicine. Quinine, however, is rather different and occupies a special place. For 300 years this was the only specific treatment for malaria. The story of its discovery, the important part which quinine has played in the colonization of the tropics, its role in both World Wars and during the Vietnam war, and the present come-back of this product all make it unique. At present quinine and related products are used in the treatment of *P. falciparum* malaria, as an antiarrhythmic, as a muscle relaxant and as a flavouring (Schweppes!)[1]. There are also some minor applications such as the treatment of babesiosis. Quinine is obtained from the bark of Cinchona trees.

In 1934 resoquine was discovered by the German H. Andersag. Only after the allies took North Africa there was renewed interest in the product. It was renamed chloroquine. Preparation in the laboratory was also economically viable. It quickly became the first choice agent and quinine was pushed into the background. In 1950 in Brazil, Mario Pinotti introduced the strategy of adding chloroquine to cooking salt (as was also done with iodine).

The synthetic preparation of primaquine was perfected after the war. The British war programme led to the development of proguanil, which itself served as a model for the development of pyrimethamine. Pyrimethamine in combination with sulphadoxine was introduced in 1970 under the name Fansidar®. After World War II it was hoped that malaria would be definitively eradicated. The use of chloroquine and the world-wide campaign to eradicate malaria by the World Health Organization, led initially to a considerable reduction in malaria infections all over the world. After the anti-malaria campaign vanished due to various circumstances, the resistance of *Anopheles* to various insecticides and the development of chloroquine-resistant and multi-resistant *P. falciparum*, malaria once more became one of the major problems.

Whereas World War II led to the discovery of some new anti-malaria agents, the Vietnam war stimulated a huge programme for the discovery of new drugs. The Walter Reed Army Institute of Research of the United States army investigated thousands of constituents. This research resulted in mefloquine (Lariam®) and halofantrine (Halfan®). Research in China produced artemisinin, pyronaridine and benflumetol.

# Treatment overview

P. vivax life cycle and site of action for different antimalarials. Source: Quique Bassat, PLoS Neglected Tropical Diseases 5(12):e1325, dec 2011

Broadly speaking, anti-malaria drugs can be divided into four major classes

- Blood schizonticides
- Antifolates
- Antimitochondrials
- Redox process-based agents

# Blood schizonticides

When the malaria parasite leaves the liver and penetrates an erythrocyte, it can begin a haemoglobin diet. Chloroquine, quinine, mefloquine and halofantrine interfere with the detoxification of haemin in the digestive vacuole of the parasite, so that haemin can generate free radicals and parasitic membrane damage follows. It is therefore logical that the drugs are not active against the parasitic

stages which precede the blood forms (sporozoites, liver forms) and which do not consume haemoglobin.

# Antifolates

Folic acid is an important metabolic factor. Humans obtain this vitamin from the food they eat. The malaria parasite must produce it for itself. Para-aminobenzoic acid (PABA) is used at an early stage of the biosynthesis of folic acid by the enzyme dihydropteroate synthetase. This step is inhibited by structural analogues of PABA, such as sulphonamides and sulphones, e.g. sulphanilamide, sulphadoxine and dapsone.

The next synthesis step is catalysed by dihydrofolate reductase. This step is prevented by pyrimethamine, trimethoprim and cycloguanil (prodrug = proguanil), to such an extent that tetrahydrofolate – the end product – is not formed. The combination of these two sequential inhibitors forms the basis of Fansidar® (similar to cotrimoxazole). Resistance to both antifolates easily develops. A specific point mutation in each gene (dhps and dhfr) is sufficient.

# Antimitochondrial products

Although artemisinin derivatives and 8-aminoquinolines (primaquine and tafenoquine) cause mitochondrial swelling, this organelle is not their chief target. Some antibiotics such as tetracycline and clindamycin prevent protein synthesis by mitochondrial ribosomes (these are similar to the ribosomes found in bacteria). They are slow-acting.

Atovaquone is a naphthoquinone which specifically destroys the electron transport chains of Apicomplexa. The molecule is similar to ubiquinone (coenzyme Q) which plays a role in the energy transfer between cytochrome B and C1. The enzymes of *Plasmodium falciparum* are 1000 times more sensitive to atovaquone than the corresponding enzymes in humans. Resistance can easily develop if used in monotherapy.

# Redox reactions

Primaquine and tafenoquine exercise their action via redox-active quinone metabolites. They are selectively toxic for the pre-erythrocytic stages and are the only medicaments which kill hypnozoites. Tafenoquine has in addition a pronounced blood schizonticidal action.

# Current treatment of malaria

A summary of the WHO recommendations in 2020 is provided first in these notes for clarity. For detailed dosages and special groups, see additional information in "Guidelines for the treatment of malaria: WHO; third edition, 2015.

All drugs used currently or in the recent past are described in detail below the summary.

- Complicated malaria (whatever the species, and also in all risk groups)
  - First choice: Artesunate IV (2.4 mg/kg in adults and children > 20 kg; 3 mg/kg in children < 20 kg)
  - Second choice (only if artesunate not available): quinine IV (see dosage below)
- Uncomplicated malaria (whatever the species)
  - Artemisinin-based combination treatment (ACT); five ACTs are currently accepted; all are in fixed-dose combination (FDC) nowadays and consist of 3-day regimen:
    - Artemether-lumefantrine
    - Artesunate-amodiaquine
    - Artesunate-mefloquine
    - Artenimol (dihydroartemisinin)-piperaquine
    - Artesunate + sulfadoxine-pyrimethamine (SP)

NB1: In low-endemic countries, a single dose of primaquine (0.25 mg/kg) should be added at the end of the ACT to decrease transmission (no need of G6PD determination)

NB2 : Chloroquine (see dosage below) is a good alternative for uncomplicated *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* in areas where no resistance is reported

NB3 : Primaquine (30 mg/day for 14 days) should be administered in case of *P. vivax* and *P. ovale* infections, after determination of the G6PD activity (alternative regimens available in case of low activity)

# Anti-malaria drugs

## Qinghaosu and Artemisinin derivatives

Artemisia annua in Vietnam. This plant is harvested in order to extract artemisinin from the leaves. Copyright Charles Lugt (with special thanks to prof Kager).



Artemisia annua in Vietnam. This plant is harvested in order to extract artemisinin, used for malaria treatment. Copyright Charles Lugt (with special thanks to prof Kager).

Artemisinin and its derivatives have become essential components of antimalarial treatment. ACTs are now recommended by WHO as the first-line treatment for all falciparum malaria in malaria endemic countries. These plant-derived peroxides are unique among antimalarial drugs in **killing the young intra-erythrocytic malaria parasites**, thereby preventing the more pathogenic mature stages. Huang hua hao or qinghaosu ("essence of qinghao") originates from a Chinese plant, Artemisia annua (sweet wormwood). The antimalarial properties of the traditional Chinese medicine qinghaosu were discovered and developed by Chinese scientists in 1971 (secret "project 523"). This research effort was prompted by the requests of Ho Chi Minh to Zhou En Lai for antimalarial drugs for the Vietnamese troops (cfr the efforts of the American forces to develop halofantrin and

mefloquin).

Artemisinin has the derivatives artesunate (the hemisuccinate; -CO(CH2)2COOH), arteether (the ethyl ether; -OCH2CH3), artemether (the methyl ether; -OCH3) and the reduced substance artenimol, syn. for dihydroartemisinin. Their plasma half-life is very short: 1 hour, both in healthy volunteers and in patients with active malaria.

Artemisinins are not active upon liver stages, but upon both the immature sexual and the all asexual blood stages. Their broad stage specificity (as opposed to quinine) has several therapeutic consequences. Killing young circulating ring-shaped trophozoites results in a more rapid reduction in parasitaemia as compared to other antimalarials and reduces the number of parasites that mature and sequester in the post-capillary venules. Quinine does not stop sequestration since it acts on the mature parasite stages, which have already adhered to the vascular endothelium. Since artemisinin reduces the number of gametocyte carriers, it helps to prevent malaria transmission, although artemisinin does not kill mature gametocytes of *P. falciparum*. In low-transmission areas, where symptomatic infection constitutes the main source of transmission, ACTs reduce gametocyte carrier rate, and if widely employed is expected to reduce the incidence of malaria. Artemether, artesunate and dihydroartemisinin reduce the number of parasites by a factor of approximately 10,000 for each asexual cycle. After two cycles (3-day treatment) there is a $10^8$-fold reduction of the parasitaemia. The longer acting partner drug will then eliminate the remaining low numbers of parasites.

The medication is best avoided during the first trimester of pregnancy, if a good alternative is available (quinine + clindamycin). Recent large studies (PREGACT) have demonstrated the safety of ACT administered during the second and third trimesters on pregnancy and infant outcome. In observational studies of pregnant women treated with artemisinin derivatives during the first trimester, no differences were noticed in the risk of miscarriage, stillbirth or congenital anomalies when compared to quinine treatment. Although data are limited, the use of ACTs is probably safe throughout gestation, especially if alternatives are not available.

Artemether (Paluther®, Arteminth®, Cotexcin®, Artenam®) is an oil-soluble derivative that can be used for IM administration.

Artesunate (Artenam®, Artesunate®, Arsumax®, Artemax®, Arinate®, Plasmotrim®) is the fastest-acting artemisinin derivative. It can be administered parenterally (IV, IM), rectally or orally. For IV use the dose is 2.4 mg/kg as start dose. This dose is repeated at least after 12 hours and 24 hours. The side-effects are mild and are difficult to distinguish from the effects of malaria itself. However, delayed onset haemolytic anaemia has been observed in about 20% of travellers who receive

artesunate after about 2 weeks. This post-artesunate delayed haemolysis is also described in endemic countries. Haemoglobin monitoring 1 and 2 weeks after artesunate administration is strongly recommended for this reason, particularly after an episode of severe malaria.

There is now strong pharmacological and clinical evidence that artesunate is superior over quinine for treating severe malaria (35% reduction of fatalities in Asian adults and 22% reduction of fatalities in African children). If patients with severe malaria cannot be treated orally and transport to a hospital for IV therapy will take more than 6 hours, a single inexpensive artesunate suppository at the time of referral substantially reduces the risk of death or permanent disability. A single dose of artesunate, given rectally (by e.g. parent), can provide parasiticidal blood concentrations within 10–20 min and can already halve parasitaemia numbers within 6–12 h.

Artenimol (more commonly named dihydroartemisinin) is obtained by reduction (hydrogen addition) of artemisinin. Together with piperaquine it is available as a fixed drug combination known as Eurartesim®. Artenimol has a short half-life, as opposed to piperaquine which has a long half-life.

After a decade of use in monotherapy in Southeast Asian countries, it has become clear that monotherapy would quickly lead to resistance to artemisinin derivatives (5-10% recrudescence after 7 days of monotherapy). Since 2005, to protect this "last-line" drug, WHO has strongly recommended to systematically combine artemisinin with another, partner drug with a longer half-life to treat all falciparum malaria in endemic countries. "Accepted" partner drugs are amodiaquine, pyrimethamine/sulphadoxine, lumefantrine, piperaquine or mefloquine (see other drugs). New ACT compound are emerging such as pr

Artemisinins also have some activity against other parasites, for example they kill the young stages of trematodes such as schistosomes and Fasciola. They are studied also in animal models of clonorchiasis.

## Lumefantrine or Benflumetol

Lumefantrine (= benflumetol) was registered in China in 1987 for the treatment of *P. falciparum* malaria. The half-life in the blood is approximately 4 days. The product is not active on the liver stages or gametocytes. Lumefantrine, like chloroquine, probably destroys heme polymerization (a detoxifying pathway for the parasite). It is synergistic with artemether. The combination artemether-lumefantrine is known as co-artemether (AL; Riamet®, Coartem®: artemether 20 mg/lumefantrine 120 mg, adult dose 2×4 tablets/d for 3 days). The combination artemether-lumefantrin is probably the most used ACT worldwide.

The possibility of drug-interaction and QTc-prolongation needs to be studied further, especially if this product would be used as stand-by medication in travellers to the tropics who also might take certain quinolones, azoles, macrolides or prokinetics (domperidone).

Absorption in the intestine is highly variable from person to person and is greatly increased (up to 16-fold) by fatty food. Since people who are ill generally do not eat much, this has important consequences. Early in the treatment very little lumefantrine is absorbed. In combinations, such as Co-artem, the artemether is responsible for the initial important reduction in the number of parasites and the low residual numbers of parasites is then cleared up by lumefantrine.

In HIV-infected children, lopinavir-ritonavir-based ART (Kaletra) was associated with a decreased incidence of recurrent malaria (reinfection) as compared to an NNRTI-based regimen, largely because of an interactions that increases drug levels of lumefantrine.

## Piperaquine

Piperaquine is a Chinese synthetic drug belonging to the bisquinolines. Half-life of piperaquine is 9 days. Piperaquine is a highly lipid-soluble drug. The combination dihydroartemisinin (artenimol) 40 mg with piperaquine 320 mg per tablet (Artekin®, Eurartesim®, Duo-cotecxin®, adult dose: 1×4 tablets/day for 3 days) is increasingly used in first-line in many endemic countries.

In 2006 Papua New Guinea became the first country to implement dihydroartemisinin-piperaquine treatment for *P. falciparum* and *P. vivax* infection in pregnant women during the second and third trimesters as well as its first-line therapy for any case of malaria. Because of the slow elimination of piperaquine, this treatment provides up to 6 weeks posttreatment prophylaxis against new infections and relapsing *P. vivax* infection (better than all other ACTs). It is recommended in travel medicine to check first an ECG to exclude an underlying QTc prolongation in people with serious liver, kidney or heart diseases or in people taking other QTc prolongating medication (macrolides, fluoroquinolone, domperidone, …). It is contra-indicated if > 500 msec and to be used withcaution if QTc > 450 msec.

## Amodiaquine

Amodiaquine is closely related to chloroquine. Long-term use causes grey skin pigmentation in white people. Sometimes there are severe side effects (agranulocytosis in approximately 1/2000, liver toxicity in approximately 1/15,000). Amodiaquine (Camoquine®, Flavoquine®, Malarid®) had been rarely used in monotherapy. There is therefore less resistance to amodiaquine than to chloroquine.

Since the product is eliminated slowly, a single dose of 600 mg was (and is) sufficient.

Amodiaquine is nowadays the partner drug of artesunate in one of the 5 recommended ACTs. This therapy exists now in fixed-drug combination (Coarsucam®, ASAQ: 100 mg artesunate/270 mg amodiaquine, adult dose: 1×2 tablets/d on 3 consecutive days) and because of its low price, has become the first-line ACT for P. falciparum in many African countries.

## Mefloquine

Mefloquine (Lariam®) is a long-acting product. After 2 to 3 weeks half of the dose is still present in the body. Mefloquine has a rather slow onset of action. For curative use, mefloquine is always combined with other antimalarials, and its use in monotherapy for treatment is now strongly discouraged (major side effects, while effective alternatives exist). The combination mefloquine + pyrimethamine + sulphadoxine is known as Fansimef®. Now, mefloquine is used with artesunate in a fixed-drug combination and is one of the first-line therapies of Pf malaria in many countries: artesunate 100 mg/mefloquine 220 mg (ASMQ), 1×2 tables/d for 3 consecutive days (adult dose).

Mefloquine plays an important (although decreasing) role in prophylaxis: cfr. infra.

## Quinine

Quinine has long been a first line anti-malarial drug and was for a long time one of the only parenteral treatment options. More recent studies however, showed clinical benefit of parenteral Artesunate and oral artemisinin combination treatment over quinine, together with less side effects. Quinine is still a powerful product, which acts upon the schizonts of the parasites in the blood (it is a schizonticide). It thus acts **chiefly in the second half of the maturation cycle**: on the parasites which are sequestered in the small blood vessels (not on the young ring forms in the peripheral circulation). Quinine also possesses gametocytocidal activity against *P. vivax, P. malariae* and *P. ovale* (but not against gametocytes of *P. falciparum*). As for chloroquine, quinine causes an inhibition of hemozoin biocrystallization in the heme detoxification pathway, which facilitates the aggregation of cytotoxic heme. Free cytotoxic heme accumulates in the parasites causing their deaths.

Quinine sulphate is administered orally. It is absorbed well in the intestines. Quinine bihydrochloride is injected, preferably by slow IV (infusion with glucose because of the risk of hypoglycaemia). IM injections may lead to sterile abscesses but can be used where necessary if there are no alternatives available. For IM injection, it is best to use a diluted solution (60 to 100 mg/ml) instead of the

concentrated solution (300 mg/ml). Quinine administered via IM injection is absorbed well even in severe malaria. Treatment with quinine is unpleasant (bitter taste, cinchonism) and poor compliance after the acute phase is common.

## Treatment regimens

The basic regimen is 10 mg salt/kg, every 8 hours, orally or slow IV. Currently, a loading dose of 20 mg/kg IV over 4 to 8 hours is universally recommended for the first administration (followed by 10 mg/kg every 8 hours). This should be continued for at least 4 days, preferably 7 to 10 days (if used in monotherapy). This is an unpleasant treatment. Because there is still a risk of relapse if quinine is used in monotherapy even for > 7 days, another product is generally combined with it, e.g. tetracycline or clindamycin. This allows also to shorten the quinine administration to 4-5 days. Sometimes treatment with Fansidar® is given after a few days, which shortens the treatment period, but only in regions where this drug is still sufficiently effective. If a patient vomits within an hour after swallowing the medication, the whole dose should be repeated. If vomiting occurs longer than one hour after ingestion, no new dose is necessary. In case of repeated vomiting IV administration is required.

## Side effects of quinine

Quinine is a substance with highly irritating properties (also for the gastric mucosa: nausea is not uncommon). Capsules are therefore best taken after a meal. Quinine increases the secretion of insulin from the pancreas, increasing the risk of hypoglycaemia. Quinine allergy is not common. What is common is a range of side effects such as tinnitus, temporary deafness for high frequencies, headache, nausea and palpitations. These toxic phenomena are known as cinchonism: quinine was first isolated from the bark of the cinchona tree. This reduces the patient's compliance.

Quinine increases irritability of the pregnant uterus. In case of need one must not hesitate to use quinine in a pregnant woman with malaria (malaria itself can lead to abortion, preterm labour or death in utero). To prevent an impending premature labour, a tocolytic agent can be given such as the beta 2-mimetic ritodrine, fenoterol or salbutamol. The calcium antagonist nifedipine is as effective a tocolyticum as the beta-mimetics. Prolongation of the PR, QRS and QT intervals may occur during the use of quinine (as with quinidine). If the patient has atrial fibrillation, conversion to sinus rhythm may occur with possibly arterial embolic complications. Atrial fibrillation which has already been present for more than 48 hours is a contra-indication for quinine. Congenital long QT syndrome and Brugada syndrome are equally formal contra-indications for using quinine. ECG monitoring to detect

QTc-prolongation is recommended during quinine therapy, especially in case of kidney failure.

Overdose of quinine may lead to very severe situations such as deafness, delirium, bradycardia, hypotension, respiratory arrest or death (lethal dose approximately 8 gram). Overdose may also lead to blindness via a direct toxic effect on the retina and possibly also due to spasms of the retinal blood vessels and subsequent retinal ischemia.

### Quinine and Gin Tonic

Unlike the majority of other bitter products which occur naturally, the bitter taste of quinine is short-acting with no annoying after-taste. It is therefore used as a flavouring to produce tonic water. The British colonialists in India often drank gin and tonic. The present-day tonic water contains approximately 15 mg per litre, however, only enough to give a bitter taste. Copious drinking of gin and tonic in order to prevent malaria, is thus only an excuse for drinking gin.

### Why is quinine resistance still rare?

The product has been used for more than 360 years. This is in stark contrast to the resistance to other malaria drugs or antibiotic resistance in bacteria where the "useful life" of a product is measured in years or a few decades. The concept of a standard dose was only developed in the twentieth century. Earlier the duration of treatment and the dosage were left to the discretion of the doctor. This together with the fact that the concentrations of alkaloids varied greatly from plant to plant and that quinine was never pure, meant that malaria was treated with a therapy which must have produced the most varied blood levels. Yet no wide spread quinine resistance has been reported. The answer to the question why there is virtually no quinine resistance, could be very important. Is the target molecule of quinine so special that mutation is not possible? It would then be very helpful to know this target. It could also be that there is quinine resistance, but that it was not, and has not been recognized. However, this is doubtful. Is it that the present recommended dose is much higher than that which was formerly necessary? Is it the fact that "quinine" is actually a mixture of various active products, which prevents resistance developing? Resistance to combined therapy requires multiple, simultaneous mutations which is less readily achieved than that to single products. It is possible that quinine has not previously been used at levels which create sufficient evolutionary pressure. The majority of malaria cases in Europe and America were P. vivax infections. Even in British India, P. vivax represented the lion's share of infections. In P. falciparum endemic regions, only a few fortunate people were able to take quinine and then only when they had to (because of unpleasant side effects). Few used quinine as a prophylactic agent

(especially among the indigenous population). What is more, quinine has a short half-life, so that the parasite was only exposed to subtherapeutic concentrations for a short time. Probably its limited use is the reason for the absence of resistance, but if used on a larger scale, quinine resistance may yet become a reality in years to come.

# Chloroquine

Despite the presence of this resistance, chloroquine still has a place in treatment. It is still active against non-falciparum plasmodium species almost everywhere and could theoretically still be used against chloroquine-sensitive *P. falciparum* in very limited areas: Central America and the Caribbean. Elsewhere, chloroquine is not recommended any more against *P. falciparum* even in immune patients, who do not usually appear very ill.

The trophozoite in the red blood cell breaks down haemoglobin using lysosomal enzymes. In this digestive process ferriprotoporphyrin IX (haemin) is formed, which is toxic to the parasite and is usually polymerized to non-toxic malaria pigment. Chloroquine binds to ferriprotoporphyrin IX and prevents detoxification.

Since the liver parasite do not feed on haemoglobin this drug is not active at the pre-erythrocytic stages of *Plasmodium sp.*

Chloroquine is available in tablet form as chloroquine sulphate (Nivaquine®) and as chloroquine diphosphate (Resochine®). Hydroxychloroquine sulphate (Plaquenil®) is different and is used in e.g. rheumatoid arthritis, lupus erythematosus and Q-fever. The injectable form is chloroquine dihydrochloride. Nivaquine® tablets contain 100 mg chloroquine, but availability of this drug has decreased over the last years.

Chloroquine is a powerful schizonticide. It has strong affinity for various tissues and organs. It is fast-acting and remains in the blood for many days. A brief treatment (3 days) is therefore possible.

Chloroquine may be given orally, SC, IM or SLOW IV (infusion). Never inject an ampoule of chloroquine IV rapidly as a bolus or rapid infusion (fatal arrhythmia). The injections are not painful.

There are several different treatment regimens. Most of the time it is given orally, 25 mg/kg spread over three days. Parenteral administration should be discontinued as soon as oral administration is possible.

Chloroquine is cheap and not very toxic in normal use.

- Some people are allergic (pruritus, rash) or suffer nausea.
- People with psoriasis are more at risk of side effects.
- A reversible precipitation of chloroquine in the cornea may occur, resulting in small opacities. This may result in seeing haloes around objects, blurred vision or photophobia. This form of keratopathy may become manifest quite rapidly (a few weeks after beginning treatment). After discontinuing the medication it is completely reversible.
- Chloroquine accumulates in melanin-containing tissues. Chronic use may lead to abnormalities of the choroid and retina (chorioretinitis). This toxic retinopathy is not reversible. The abnormalities are always bilateral and symmetrical. Often there is maculopathy (bull's eye lesion) with central and paracentral scotomata, but constriction of the peripheral field of vision may also occur. The total cumulative dose before such problems occur is generally 100 gram chloroquine or more.
- Chloroquine has a narrow safety margin (just 30 mg/kg may be fatal). In case of overdosage myocardial depression, hypotension and severe arrhythmias may occur. ST-segment abnormalities and T-wave inversion occur. Broadening of the QRS complex (>0.12″) and ventricular arrhythmias have a poor prognosis. The patient may become comatose, vomit and aspirate stomach contents. In acute intoxication diazepam is given (Valium® 1 mg/kg) and adrenalin (= epinephrine) or dopamine if these are available.

## Pyrimethamine + / – Sulphonamides

Fansidar® is a combination product of pyrimethamine 25 mg and sulphadoxine 500 mg per tablet (Mekalfin® is another commercial name). The curative treatment for an adult is 3 tablets taken as a single dose. Sulphadoxine is a long-acting sulphonamide (t½ = 8 days) which in case of allergy may cause severe skin lesions (erythema multiforme and Stevens-Johnson syndrome). *Plasmodium falciparum* has rapidly developed resistance to this product in many parts of the world. It is not used any more as monotherapy but may be combined to artesunate (at least in regions where no resistance has been observed): Sulfamon®, Artecospe adult® (AS+SP) = artesunate 100 mg + sulphadoxine/pyrimethamine 500/25 mg: 1×2 tablets AS/d for 3 days + 3 tablets SP single dose. This combined treatment is available in co-blister packs (this is not the same as coformulated tablets!). Fansidar is also widely used as intermittent preventive treatment for pregnant woman in Africa (either they present with blood parasite or not, once or twice during pregnancy) and still provide substantial benefit in preventing maternal and infant anaemia and low-weight birth (even in areas with increasing resistance). Though recent studies comparing dihydroartemisine-piperaquine (and other artemisinin-based combination therapies [ACTs]) vs pyrimethamine-sulphadoxine as intermittent preventive treatment during pregnancy (IPTp), showed that ACTs are usually superior in decreasing the malaria

burden during pregnancy. Use of ACT in IPTp is however not yet a WHO recommendation, pending results on the long-term risk of developing resistance.

## Halofantrine (Halfan®)

This is fast-acting, effective and has few but potentially lethal side effects. Given a series of casualties, it is no longer used and production has been abandoned. It has been replaced by a similar but non-toxic product: lumefantrin. Halofantrin was very dangerous in people with a long QT interval: reportedly lumefantrin does not present the same toxicity, but this deadly experience with halofantrin makes clinicians very cautious, ordering always an ECG before treatment whenever possible and almost always in high income settings.

## Primaquine

Primaquine is an 8-aminoquinoline. It is inactive upon asexual blood forms. It does have an important though only partial causal prophylactic effect (on both *P. falciparum* and *P. vivax*) but only if it is taken 24-48 hours (max. 96 hours) after inoculation with sporozoites. It acts upon the exo-erythrocytic stages of the parasites (liver schizonts). The half-life is relatively short (4 hours). For causal prophylactic use a daily dose of 15-30 mg may be taken. These regimens are not very popular and there has been little experience of them. Chemoprophylaxis with primaquine can be stopped 3 days after leaving a malarious area.

In cases of *P. vivax* or *P. ovale* malaria, hypnozoites remain in the liver after therapy with ACT or chloroquine/quinine. These may be destroyed by primaquine. In the past, 15 mg base per day was used for 14 days [26 mg primaquine biphosphate = 15 mg primaquine base], but current medical opinion favours 30 mg per day for 2 weeks (increasing tolerance of some P. vivax strains). This drug is contra-indicated in pregnant women and in people with a significant deficiency of G6PD (glucose-6-phosphate dehydrogenase), an enzyme in the red blood cells (risk of haemolysis in patient and/or fetus).

Primaquine also acts on *P. falciparum* gametocytes. Therefore, in some circumstances (e.g. refugee camps) it may be given to reduce transmission (single dose of 45 mg). It is nowadays thoroughly investigated (in low dosage) as a potential strategy to decrease/suppress transmission in low-endemic areas contemplating elimination. Detection of underlying G6PD-deficiency is however a major hurdle for its use on a larger scale. Reliable point-of-care tests to detect G6PD deficiency would remediate this problem. Several low-endemic countries have already adopted the systematic administration of

primaquine (0.25 mg/kg) at the end of the course of antimalarials/ACT administered to treat a clinical malaria episode. Mild methaemoglobinemia is often observed with primaquine but rarely with clinical consequences.

## Tafenoquine or Etaquine

Etaquine or tafenoquine is a new 8-aminoquinoline, derived from primaquine. It has a half-life of two weeks, which is much longer than the half-life of primaquine. It may be taken orally and has low toxicity. It is active against *P. falciparum* and *P. vivax*. It is an effective schizonticide and is also active on the pre-erythrocytic stages, including the hypnozoites of *P. vivax*. Screening for G6PD deficiency is also required and this is always a limiting factor in low-resource settings. Tafenoquine has been approved in 2018 for the radical (relapse-preventing) treatment of *P. vivax* and *P. ovale* malaria (single dose of 300 mg just after the treatment of the clinical episode) and for malaria chemoprophylaxis (200 mg weekly). The experience of this new drug in not yet that large in clinical practice, but it is expected that replace primaquine soon, due to its much shorter/easier administration.

## Proguanil and Chlorproguanil

Proguanil (Paludrine®) and chlorproguanil (Lapudrine®) are biguanides which are converted in the body to the active product cycloguanil.

The combination of chlorproguanil with dapsone is also known as Lapdap®. It is used as a cheap, short-half-life antifolate. It may be combined with artesunate (combination known as "CDA or Chlorproguanil-Dapsone-Artesunate"). In Malarone®, proguanil is combined with atovaquone and both drugs have a synergetic effect explaining its increased efficacy (despite the use of two drugs with moderate activity).

## Atovaquone

Atovaquone (Wellvone®, Mepron®) is a lipophilic hydroxynaphthoquinone. Atovaquone is a powerful schizonticide for *P. falciparum* and *P. vivax*. On monotherapy recrudescence occurs very quickly. To avoid this problem, it is combined with proguanil (brand name of the atovaquone + proguanil combination = Malarone®). Atovaquone/proguanil is both used in curative and prophylactic regimen. It cannot be used in renal failure because the blood levels of proguanil/cycloguanil are much higher. Simultaneous use of atovaquone/proguanil and rifampicin is not recommended (blood levels 50%

lower). Most recent data state that it's probably safe in pregnancy, even during the first trimester. The curative dose is 4 tablets of atovaquone/proguanil 250/100 mg for 3 consecutive days.

The product is also being studied in toxoplasmosis, babesiosis, leishmaniasis, microsporidiosis and in Pneumocystis jirovecii pneumonia. In the treatment of babesiosis it proved more active in some animal studies than the combination of clindamycin/quinine.

In general atovaquone/proguanil is very well tolerated. Nausea, diarrhoea and headache are the most frequent side-effects. Stevens Johnson syndrome has also been described. Resistance to atovaquone/proguanil has been rarely described even though a single mutation is enough to substantially decrease its activity. The limited use due to its high price might explain in part the lack of resistance.

## Miscellaneous products

Tetracycline, minocycline and doxycycline are antibiotics which are active against malaria parasites but are very slow-acting. For this reason, they are never given as monotherapy, but in combination with quinine. They very much reduce the risk of relapse. Doxycycline has the advantage that it only needs to be administered once daily. Doxycycline is is sometimes used for malaria prophylaxis (cfr. infra).

Clindamycin (Dalacin®) is also active against plasmodia but is a second choice drug (risk of pseudomembranous colitis due to Clostridioides difficile). It is given together with quinine for Pf attack during pregnancy.

# Drug resistance

In chloroquine-sensitive *P. falciparum* the drug is concentrated in the parasite. There is slow outflow (t½ = 50 minutes) of chloroquine from the sensitive parasite. In resistant parasites t½ for outflow = 1 to 2 minutes. Resistance is thus not due to inactivation, breakdown or neutralization of chloroquine. The parasite quickly pumps the product away to the blood, so that the concentration of chloroquine within the parasite is low. At present this cannot be counteracted in humans (in vitro reversible with verapamil).

PCR technology [polymerase chain reaction] is required to differentiate a recrudescence (or relapse) in an endemic region from a re-infection with the same species. Several polymorphic loci are

analyzed. Every combination of alleles that is tested, is rare and permits differentiation between strains.

The first signs of chloroquine-resistant P. *falciparum* infections occurred in the '60s, more or less simultaneously in Colombia and Thailand (countries which mixed chloroquine in commercial table salt). This resistance spread progressively and is now a significant problem in most continents. There are three grades of chloroquine resistance (RI, RII, RIII). In RI the parasitaemia after therapy is so low that it falls below the detection threshold, to rise above it again within 28 days. In RII the parasitaemia is reduced by at least 75 %, but the parasites remain detectable in the peripheral blood throughout the treatment. In RIII chloroquine has no effect on the parasitaemia.

In 1991 chloroquine-resistant P. *vivax* strains were discovered in Papua New Guinea. In 2006, already 65% of the Papuan P. *vivax* strains were chloroquine resistant. P. *vivax* chloroquine resistance in other areas, such as Indonesia, India, Brazil, Guyana is spreading. Chloroquine is not the first-choice treatment any more for P. *vivax* in Indonesia and neighbouring islands. To date resistance to chloroquine in P.*ovale* is very rare. The first chloroquine-resistant P. malariae has been reported (Malaysia, 2002).

Resistance also developed against other drugs, including Fansidar®. The situation is evolving rapidly and is a serious threat to the future use of the artemisinins. The highest resistance against anti-malarial drugs is found in some regions in Southeast Asia, including Cambodia, the Thailand-Cambodia border and the Thailand-Myanmar border. It is only a question of time before these resistant strains spread further geographically. There are several reasons for this increasing resistance. Important factors include inadequate individual patient compliance, treatments that are often discontinued prematurely, frequent underdosing, earlier mass-treatment campaigns reaching only part of the population and therapy being sometimes only partly administered, as well as the use of chloroquinated salt [Cambodia, Brazil (the Pinotti method)]. Among the causes of the swift increase in geographical spread are the large-scale migrations of today, and the ability to move rapidly from place to place. Some products are eliminated slowly from the body (e.g. mefloquine t1/2 = 2 weeks) so that for some weeks a subtherapeutic level of the product is present in the body (as what happened with the addition of choloquine to the salt). When malaria parasites are exposed to such low concentrations, partially resistant strains have a selective advantage. The occurrence of subclinical cases (premunition) functions as a source and reservoir for transmission of parasites with reduced sensitivity. Since the cost price of alternative drugs is generally higher than that of traditional treatments, under-dosing with new drugs will become even more important in future.

On the other hand, reversion of chloroquine resistance has been described in areas where chloroquine

was not routinely used for several years. In Malawi, treatment of uncomplicated malaria with chloroquine was 99% effective 12 years after withdrawal due to resistance. This opens possibilities for the use of chloroquine as a partner drug in combination treatments. Stricter control on "fake" drugs (counterfeited medication), some of which contain small amounts of active material will be an essential component in health programs. One idea to combat counterfeit drugs and piracy is to tag individual genuine medication boxes with an authentication number (itemunique code) under a scratch-off label on the wrapping. When revealed just before purchase, this number can be sent toll-free by telephone text message to an independent certifying company (e.g. Sproxil) which then instantly and automatically replies.

In 2008 resistance to artemisinin has been documented for the first time in Cambodia (borders with Thailand and Vietnam) and in the years after it was described in Thailand, Vietnam, Laos and Myanmar. Treatment failures rates after artesunate-mefloquine and artemether-lumefantrine often exceed 10% in these areas, which is worrying, and higher than anywhere else at present. Full resistance seems to be adequately predicted by a failure to clear the parasites after 3 days of combined treatment. This delay in parasite clearance is the best early marker of resistance so far and is therefore scrutinized in all these regions. Resistance to artemisinin is associated with mutations in the kelch protein gene on chromosome 13 (kelch13). It is worrisome that in some regions in Southeast Asia decreased sensitivity to the artemisinin partner drug was found in combination with the kelch13 mutation. High MIC's (minimal inhibitory concentration) to piperaquine in Cambodia lead to recrudescence in dihydroartemisinin-piperaquine treated patients. Treatment in this region should therefor consist of mefloquine plus artesunate. In 2019, a 15% treatment failure was noted among patients treated with artesunate-sulfadoxine-pyrimethamine due to a mutations in the kelch13 gene combined with resistance to sulfadoxine-pyrimethamine.

In general, six-day courses of ACT still appear to be efficacious if three-day treatments are failing.

Outside of Southeast Asia, only a few cases of artemisinin resistance have been described in Guyana. In Africa, kelch13 mutations where found but it is not clear whether these mutations confer artemisinin resistance. Clinical failure of ACT has not been described in Africa. Ongoing worldwide molecular surveillance and assessment of the efficacy of ACT regimens are warranted to detect resistance and its spreading early.

# Malaria – Prevention

## External agents

Anopheles mosquitoes only bite in the evening and at night. It is possible to protect oneself by wearing protective clothing and using an undamaged mosquito net. Effectiveness is increased by treating the net with pyrethroids (insecticides) such as permethrin (Permas®, Peripel®), lambda-cyhalothrin (Danger®, Demand CS®, Matador®) or deltamethrin (K-Otrine®). This will increase further in importance in the future. In most instances, permethrin will be augmented by piperonyl butoxide. Piperonyl butoxide is the most widely used synthetic pyrethrin synergist and there are no reports available on toxic effects on humans resulting from the exposure to it. Piperonyl butoxide is not an insecticide itself but a cytochrome P450 inhibitor which allows pyrethroids such as permetrin to be much more active (10x). Inhibition of the detoxification pathway allows higher unchanged systemic concentrations of the active insecticide to remain within the target animal for a longer period. This is here now.

### Long-lasting insecticide treated nets

Mass produced long lasting insecticide treated nets (LLINs) are replacing older style bed nets. Olyset net was the first LLIN which became commercially available. Sumitomo's Olyset® technology incorporates permetrine insecticide directly into polyethylene filaments which can be woven into sturdy bed nets to provide long-lasting protection from night-time biting mosquitoes. Olyset Plus, which received WHO approval in July 2012, retains the controlled-release technology and durability, and contains 2% permethrin and 1% of the synergist piperonyl butoxide (PBO). The fibres have been designed to release the two ingredients at a constant ratio of 2:1. The 'bleed rate' at which permethrin and PBO migrate from the internal reservoir in the fibres to the surface of the net has been adjusted in order to make the net active again within 1-2 days of washing. For this work, Sumitomo Chemical became the co-winner of the 2012 'Application of Core Competence' category – Global Business Coalition Health Award. A major production plant has been set up in Tanzania.

Fine-mesh gauze can be applied to windows and ventilation shafts. One good argument for using a mosquito net is the fact that it also protects from nuisance insects such as *Culex* mosquitoes and bedbugs. In regions where there are few *Culex*, people are not so ready to use a net: after all they cannot see or hear any mosquitoes (anopheline mosquitoes fly with little noise).

Insecticides based on pyrethrum can be dispersed by means of spraying (spray gun), evaporation (heated electric plate) or burning (mosquito coil, e.g. with esbiotrin). Insecticides can also be applied to the walls or to the curtains by the windows.

There are also various insect repellents. DEET (N,N-diethyl-m-toluamide, now called N,N-diethyl-3-methylbenzamide) is moderately active and can be applied as an alcoholic solution to the skin. This produces a sticky effect when the alcohol evaporates. The effectiveness is only moderate. DEET is absorbed through the skin and is eliminated quickly via the urine. There is no accumulation in the body. The higher the concentration, the longer the duration of action: DEET 20-30% gives 4-6 hours protection, DEET 50% offers 8 hours protection. Concentration higher than 50% don't give significant longer protection.

Alternative repellents are (p)icaridine(Care-Plus®Repel-it; Parazeet®) and IR3535 (Cinq sur Cinq®, Moustidose®).

## Intermittent preventive treatment (IPT) and seasonal malaria chemoprophylaxis (SMC)

In highly endemic countries (sub-Saharan Africa), several "preventive" strategies have been promoted and adopted for special risk groups or for some periods of higher transmission. They consist of administering some drugs with antimalarial activity at regular intervals to a group of population with no previous diagnostic testing for malaria. The main aim is to control the malaria morbidity and important reductions of clinical and severe malaria or malaria-related complications (on fetus/newborns for example) have been repeatedly demonstrated.

At this moment, IPT use is recommended by WHO

- in pregnancy (ITPp) as part of antenatal care: sulfadoxine-pyrimethamine (SP) starting from the second trimester with at least three administrations at one-month intervals minimum
- in infants (< 12 years) during the immunization program: SP (where still effective) at the second and third rounds of vaccination against tetanus/diphtheria/pertussis and at vaccination against measles
- in children (< 6 years) in the sub-Sahel region during the rainy season: SP + amodiaquine once a month during each transmission season (strategy called SMC)

On an important prospective note, ACTs are also increasingly explored as IPT in various populations for preventive purposes. ITP with ACT is currently investigated in pregnant women, infants, children <

6 years, school-age children, whole population where malaria is about to be eliminated. This field and the related WHO recommendations are expected to evolve deeply in the coming years

# Chemoprophylaxis for travelers

Chemoprophylaxis is in the first instance intended as prevention of *P. falciparum* malaria. No single drug which is taken preventively is 100% active against sporozoites and no single drug prevents the formation of liver forms (except primaquine). While taking prevention no vivax or ovale malaria will occur but after they have been discontinued an attack with these plasmodia is possible in the following months or years.

In view of the extensive resistance of *P. falciparum*, at present no 100% satisfactory protection against this latter parasite is possible. Advice as to whether or not to take medication and which kind of drug to take, will depend on the region and differ from person to person (short journeys, resident, local population, pregnancy, young children, allergy, chronic diseases, use of other drugs and so on). Recommendations vary from country to country and evolve in time.

- In regions with only *P. vivax* and/or sensitive *P.falciparum*  (WHO type A) chloroquine 300 mg/week will suffice.
- In zone C with resistant/multidrug resistant *P. falciparum*, 3 different regimens are currently recommended:
- Atovaquone/proguanil 250/100 mg 1 tablet per day beginning 1 day before departure until 7 days after return
- Doxycycline 100 mg/day during the stay and up to 4 weeks after return
- Mefloquine 250 mg 1 tablet per week, to start two-three weeks before departure, and to continue up to 4 weeks after return

The decision should be individualized, since it depends on several aspects (side effect profile, type of trip, budget). Given the lower cost of generic drugs of atovaquone/proguanil and its good tolerance, atovaquone/proguanil is often chosen as the prophylactic treatment, especially for shorter journeys.

Doxycycline is an alternative in case of atovaquone/proguanil intolerance., Prolonged ingestion of doxycycline can lead to phototoxicity, including photo-onycholysis. Sunscreens do not block ultraviolet A well enough to prevent phototoxic reactions to doxycycline.

Today, the use of mefloquine as preventive treatment has decreased. The plasma half-life of mefloquine is 2 to 3 weeks. Ingestion of 1 tablet per week produces stable blood levels after 7 weeks.

Traditionally it is said that mefloquine prophylaxis should be started before departure. This guideline is based on the consideration that intolerance to the drug can be monitored in this way. It is safe to begin the medication 15 days before departure so that 3 tablets are taken before leaving. In this way 75% of the side effects can be detected. At the prophylactic dosage (adults one 250 mg tablet per week) side effects occur in 2 to 3% of people, which require that the prophylaxis be discontinued. Rarely (1 in 12,000 to 15,000) preventive dosages may trigger epilepsy or psychosis may occur. Epilepsy and arrhythmias (including the use of beta-blockers, calcium antagonists and digitalis) are contra-indications for the use of this product. Latest data indicate that it is proven safe during pregnancy. There are sufficient data that it is safe if taken for longer periods. The first case of mefloquine resistance was described in Thailand in 1982. There is already mefloquine resistance on a small scale in many countries, but this can be significant locally: e.g. the cure rate in East Thailand was only 41% in 1993. *P. falciparum* malaria can thus sometimes occur in spite of correct prophylactic use of mefloquine. Mefloquine does not kill sporozoites and liver parasites (therefore *P. vivax* and *P.ovale* malaria are still possible after leaving an endemic zone and after discontinuing mefloquine chemoprophylaxis).

For longer stays we recommend after a period of adequate chemoprophylaxis (a few weeks) at arrival; to travel with stand-by emergency treatment (SBET) of quality, to use in case of malaria, either breakthrough under chemoprophylaxis or attack occurring later. It is of utmost importance to remain alert in case of fever even after several years of tropical stay. Malaria is always possible, even in regions of lower transmission and malaria should be investigated appropriately and treated accordingly. Emergency treatment for travellers in 2016 includes Malarone®, Riamet® or Eurartesim®.

The local population should not take chronic chemoprophylaxis and most people develop semi-immunity. There are however some high-risk groups: e.g. pregnancy, children less than 5 years and HIV patients. During pregnancy particularly in the second and third trimesters and also immediately post-partum, the immunological resistance to malaria falls. Intermittent preventive therapy in pregnancy ("IPTp") protects against maternal anaemia and low birth weight, and its use in areas in medium to high transmission is recommended by WHO (in most African programs Fansidar is used). The efficacy of IPTp is reduced in HIV-positive women.

# Vaccination

Research into a malaria vaccine is based on a number of possibilities. An immune response can be triggered against sporozoites and liver forms (pre-erythrocytic vaccines), erythrocytic forms (blood-stage vaccines) and/or gametocytes (transmission blocking vaccines). However the immune response

does not necessarily have a protective effect. A 100% effective malaria vaccine is not likely to be developed in the foreseeable future but a vaccine which leads to partial protection is being evaluated in different fields.

## RTS,S/AS01

In the early 1980s antibodies against sporozoites were used to identify the main antigen, circumsporozoite protein (CSP). The CSP is expressed on the surface of the parasite during the infective sporozoite stage.

In 1996 the first favourable results became known. A randomized and controlled study in the Gambia on 306 volunteers showed RTS,S/AS01 to provide significant protection against natural *P. falciparum* infection. The RTS,S/AS01 is a recombinant vaccine against the pre-erythrocytic stage of the parasite in which regions of *P falciparum* CSP are fused to hepatitis B surface antigen. It was developed by a public-private partnership with support from the Bill and Melinda Gates Foundation. The results of the large phase III trial that enrolled 15,459 infants was carried out at 11 clinical trial centers in seven countries (Burkina Faso, Gabon, Malawi, Mozambique, Ghana, Tanzania, Kenya) were published in 2012. In this trial 3 vaccines were given with a 1-month interval and some received a booster 20 months after the first vaccine to assess if higher immunity is maintained with a booster vaccine. Initial results demonstrated a vaccine efficacy of about 31% for both clinical and severe malaria in African children and a 26% vaccine efficacy against severe malaria However, a follow-up study over 7 years showed that these results were offset by rebound in later years in areas with high exposure to malaria parasites. In year five to seven after vaccination, the vaccinated group even had a higher risk of febrile convulsions than the control group with a possible higher risk for cerebral malaria and meningitis in areas with high exposure. Nevertheless, pilot implementation studies are currently being initiated in Kenya, Malawi and Ghana and will learn whether large-scale use of the RTS,S/AS01 vaccine may enter future malaria preventive programs.

## PfSPZ

PfSPZ is a newly developed vaccine, eliciting an immune response against *Plasmodium falciparum*. It is made of non-replicating irradiated whole sporozoites (SPZ), the parasite stage that infected mosquitoes inject during a bite. The vaccine is unique in using whole parasites as its ingredient. In healthy volunteers a strong protection was noted in lab studies with development of CD8+ T-cells producing IFNy. These T cells play a key role in the immune response to fight malaria in the liver. The difficulty with this vaccine however is that PfSPZ must be injected intravenously, that poses

challenges for mass vaccination campaigns. On top of this, it must be stored in liquid nitrogen at – 195 °C or colder. Sanaria, the developing company, is developing a robot that can dissect salivary glands of mosquitos. This step should make preparation and further development of the vaccine faster and easier.

A pilot trial that will enrol 2.100 people aged 2-50 years on the west African island of Bioko is being planned. If the first results are promising, the plan is to vaccinate another 10.000 people and ultimately all 280.000 habitants of the island. PfSPZ's efficacy in the field will inevitable be lower than in lab studies because people might have weaker responses to the vaccine due to pre-existing exposure to malaria or local strains of the malaria parasite might differ from the one used in the vaccine. But combined with conventional measures such as indoor insecticide spraying and insecticide treated bed nets, there is the hope to be able to completely eradicate malaria on the island.

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