Malaria

Elizabeth A Ashley MD,[1,2] Aung Pyae Phyo MD,[3,4] and Charles J Woodrow MD[2,4]

[1] Myanmar Oxford Clinical Research Unit, Yangon, Myanmar. Email liz@tropmedres.ac
[2] Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, UK.
[3] Shoklo Malaria Research Unit, Mae Sot, Thailand
[4] Mahidol Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Summary

Following unsuccessful eradication attempts there was a resurgence of malaria towards the end of the twentieth century. Renewed control efforts using a range of improved tools, in particular long-lasting insecticide-treated bednets and artemisinin-based combination therapies, have more than halved the global burden of disease, but it remains high with 445,000 deaths and more than 200 million cases annually.

Pitfalls in individual patient management are delayed diagnosis and overzealous fluid resuscitation in severe malaria. Even in the absence of drug resistance parasite recurrence can occur owing to high parasite densities, low host immunity and/or suboptimal drug concentrations.

At a population level, malaria elimination is firmly back as a mainstream policy but resistance to the artemisinin derivatives, their partner drugs, and insecticides present major challenges. Vaccine development continues on several fronts but none of the current candidates has been shown to provide long-lasting protection. Increased resources and unprecedented levels of regional co-operation and societal commitment will be needed if further substantial inroads into the malaria burden are to be made.

Introduction

Malaria is a vector-borne parasitic tropical disease found in 91 countries worldwide.[1] Of more than 120 *Plasmodia* species infecting mammals, birds and reptiles, only six are known to infect humans regularly. *P.falciparum* produces high levels of blood-stage parasites that sequester in critical organs in all age groups and cause severe anaemia in African children, in whom the vast majority of malaria deaths occur. *P. vivax* usually produces milder disease, but can be severe, and recurrent episodes bring significant associated morbidity. *P.malariae*, and the morphologically indistinguishable sympatric species *P.ovale curtisi* &*wallikeri* are understudied, but severity of illness is generally comparable to uncomplicated vivax malaria. *P.knowlesi* is a primarily zoonotic infection encountered in Southeast Asia that can cause severe malaria.

Epidemiology

Malaria is a disease of tropical and sub-tropical regions, having been eradicated from temperate countries steadily over the last 100 years. It is transmitted by the bite of the female *Anopheles* mosquito. Disease incidence depends on environmental suitability for local vectors in terms of altitude, climate, and vegetation, and implementation of control measures, and hence is inextricably

linked to poverty, natural disasters and war. Less common transmission routes are from mother to child, or via blood transfusion, a rare occurrence in non-endemic countries thanks to blood donor screening procedures, but a significant risk in resource-poor settings. [2, 3] Predictions as to the impact of climate-change on global malaria distribution in the future vary but have suggested the population at risk of malaria will increase, in particular in tropical highland areas. [4]

P. falciparum and *P. vivax* are the predominant species worldwide with an estimated incidence of 207 million and 8·5 million cases respectively in 2016.[1] The great majority of falciparum malaria occurs in Sub-Saharan Africa (~190 million cases) where transmission remains intense in many locations, although there is considerable variation in incidence within and between countries.[5, 6] Vivax malaria is much less common in this region because the human population is largely Duffy antigen negative (see predisposing factors below). In Asia and Oceania malaria case numbers are generally lower and proportions caused by *P.vivax* and *P.falciparum* are comparable, while in the Americas vivax malaria cases exceed falciparum by more than two-fold.[1]

Plasmodium malariae and *P.ovale* have a global distribution but incidence is low with *P.ovale* found mainly in Africa and Southeast Asia. Macaques are the natural hosts of *Plasmodium knowlesi*. In Malaysia, which has a high burden of knowlesi malaria, cases were initially misdiagnosed as *P.malariae* due to morphological similarities when examined by light microscopy.[7] The true global burden of disease is unknown; however, while this parasite is capable of being transmitted by *Anopheles dirus*, an important vector of human malaria, it is predominantly a zoonosis. Human infections with other simian malarias such as *P.cynomolgi* and *P.simium* may occur. They are assumed to be infrequent events with the caveat that routine microscopic examination may fail to distinguish these from the more common species.[8, 9]

Biology

The human phases of the malaria life cycle are shown in Figure 1. Sporozoites are inoculated by the bite of an infected female *Anopheles* mosquito. The parasite undergoes a pre-erythrocytic liver stage which typically lasts for one to two weeks before the onset of the blood stage, where serial cycles of asexual replication produce rising parasite numbers and hence human disease. A subpopulation of intraerythrocytic parasites switches to sexual development,[10] producing female and male gametocytes. [11] These distinctive transitional stages transmit malaria to the mosquito via a blood meal. Male gametocytes exflagellate in the mosquito midgut and male and female gametes fuse to form a zygote which transforms into a mobile ookinete and passes through the gut wall. The oocyst releases sporozoites which migrate to the mosquito salivary glands, completing the life-cycle.

In vivax and ovale infections, a proportion of sporozoites become dormant hypnozoites, causing relapses months or years after the initial infection.

Pathogenesis

Symptoms of malaria develop once the erythrocytic cycle produces a parasitaemia above a certain threshold (~100 parasites per microlitre). Incubation periods are typically 10 to 14 days for *P.falciparum* or *P.knowlesi*, two to three weeks for *P.vivax* and *P.ovale*, and 18 days or longer for *P.malariae*; however there is variation, e.g. some strains of *P.vivax* have a three to six month primary incubation period.[12, 13] Classical accounts describe periodic fever spikes at intervals corresponding to

the erythrocytic cycle length of the infecting species (48 hours for *P.falciparum*, *vivax* or *ovale* and 72 hours for *P.malariae*), resulting from synchronisation of developmental stages, but such patterns are now observed rarely.[12]

P. falciparum is unique in that erythrocytes containing mature parasites sequester inside small and medium-sized vessels, avoiding parasite clearance in the spleen but causing host endothelial cell injury and microvascular obstruction. Cytoadherence is mediated by *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), a clonally variant set of proteins exported to the infected erythrocyte surface and encoded by the *var* gene family. Subtypes of PfEMP1 bind to different endothelial receptors; for example those that bind intercellular adhesion molecule-1 (ICAM-1) and endothelial protein C receptor (EPCR) are associated with cerebral malaria.[14, 15] Infected erythrocytes also bind to uninfected cells (rosetting), and uninfected cells become less deformable, exacerbating microvascular obstruction.

The clinical impact of sequestration and associated endothelial dysfunction depends on the organ(s) involved. In the brain it contributes towards coma, in the lungs it predisposes to respiratory failure, and in pregnant women sequestration in the intervillous space of the placenta leads to placental malaria with the consequences of maternal anaemia, low birth-weight, preterm labour and increased risk of abortion and stillbirth.[16, 17] Placental cytoadherence is mediated by binding to chondroitin sulphate A (CSA), specifically via the PfEMP1 variant VAR2CSA, and the effects are most severe in primigravid women. [16]

Anaemia is a common feature of malaria and is typically multifactorial in origin with red cell loss as the leading cause in acute infections as the spleen filters both infected and damaged uninfected red blood cells.[18] A degree of intravascular haemolysis also occurs, which may be massive. There is also bone marrow suppression and dyserythropoiesis.

Recent investigations have found some evidence of endothelial cell activation in vivax malaria and suggested that peripheral parasite density may underestimate total biomass but research into pathogenesis is at a very early stage compared to *P.falciparum*.[19]

Clinical presentation

Malaria is separated conveniently into two disease presentations: uncomplicated and severe. Symptoms of uncomplicated malaria are very non-specific, and may include fever, chills, body-aches, headache, cough and diarrhoea, making clinical diagnosis unreliable. In non-endemic areas taking an accurate travel history in all patients with fever is the key to making the diagnosis. Thrombocytopenia may provide another clue. The differential diagnosis will vary depending on location. Once malaria is suspected the most appropriate course of action is to expedite laboratory testing (see below).

Severe falciparum malaria has specific diagnostic criteria. For rapid clinical assessment a short list of danger signs is used which includes prostration, fast deep breathing (reflecting underlying acidosis), and impaired consciousness. A comprehensive list is shown in Panel 1.

The most common manifestations of severe malaria are cerebral malaria, acute lung injury, which may progress to acute respiratory distress syndrome (in up to 25%), acute kidney injury, typically presenting as acute tubular necrosis, and acidosis. Lactic acid predominates but other acids have

been identified in adults with severe malaria, including hydroxyphenyllactic acid (HPLA), and α- and β-hydroxybutyric acids.[20] Severe anaemia (without major organ dysfunction) is a common presentation in children.[21] Other differences in disease presentation in children as compared to adults are more frequent seizures (in 60-80%), hypoglycaemia, and concomitant sepsis, and less frequent pulmonary oedema and renal failure.[22, 23] The case fatality rate of treated cerebral malaria is usually 10-20% and may reach 50% in pregnant women. [12] Coma may be profound with extensor posturing, positive pout reflex and teeth-grinding. Neuroimaging typically shows some evidence of brain swelling but this is less prominent in adults than in children, in whom brain swelling is strongly associated with a fatal outcome.[24-26] Characteristic fundoscopic findings in cerebral malaria include retinal whitening, changes in blood vessel colour and haemorrhages. Papilloedema is unusual.[26] Two patterns of acute kidney injury are described in malaria: one in severe malaria patients with multi-organ failure and the second in patients who have been successfully treated and do not have evidence of other organ involvement.[22]

Laboratory diagnosis

Confirming the presence of parasites in all malaria cases ensures species-specific antimalarial treatment and points to other illnesses in negative cases. The gold standard for malaria diagnosis remains light microscopy of stained blood films, thick films providing sensitivity and thin films allowing speciation and quantitation (Figure 2). However rapid diagnostic tests (RDTs) now predominate as the first-line investigation[27] with a wide range of devices available. Given the distribution of species, in much of Africa a *P. falciparum*-only test based on the highly-expressed histidine-rich protein 2 (PfHRP2) antigen is often used; this can remain positive for several weeks after parasite clearance because of persisting pitted (once-infected) red blood cells.[28] Elsewhere RDTs need to incorporate both an HRP2-detecting strip for sensitive falciparum detection and a pan-species strip that detects the lactate dehydrogenase enzyme of all human malarias, although this is relatively insensitive for *P.knowlesi* diagnosis. In Latin America HRP2 gene deletions mean that HRP2-based tests are unreliable,[29] and evidence is emerging that the problem may extend to Africa.[30] Very high falciparum parasitaemias can also produce negative results due to the prozone effect. [36]

The threshold of detection for these standard methods is approximately 50 parasites/µl (microscopy) and 200 parasites/µl for PfHRP2-based *P. falciparum* RDTs (several fold higher for non-falciparum RDTs). Nucleic acid amplification-based tests provide much greater sensitivity (often below one parasite/µl).[31]

Case management

The treatment of malaria, particularly that of *P. falciparum*, was revolutionised by the introduction of the artemisinin derivatives in the 1990s, a group of semi-synthetic compounds produced from qinghaosu (artemisinin), a natural product of the sweet wormwood plant (*Artemisia annua*). Artemisinins are rapidly effective, safe and well-tolerated. Their discovery by China's 'Project 523' was acknowledged by the award of the 2015 Nobel Prize to Tu Youyou.

Management of severe malaria

All patients diagnosed with severe malaria, including women in all trimesters of pregnancy, should receive parenteral artesunate without delay (Panel 2).[22] A higher mg/kg body weight dose of

artesunate is given to children weighing less than 20kg. Artesunate was shown to be vastly superior to quinine in large trials (35% (95% CI 18·5–47·6) mortality reduction in Southeast Asian adults and 22% (95% CI 8·1–36·9) reduction in African children).[32, 33] If quinine is prescribed a loading dose must be given and the second dose administered eight hours after the start of the first infusion.[34] Intramuscular artemether is another option but was inferior to parenteral artesunate for preventing malaria deaths in Asian adults.[35] Parenteral quinidine is still recommended as an alternative treatment in the United States but is associated with significant cardiotoxicity. Hypoglycaemia (blood glucose <2.2mM) is a serious complication of malaria and is aggravated by quinine therapy, especially in pregnant women. Management of hypovolaemia and acidosis requires cautious fluid replacement with crystalloids to reduce the risk of pulmonary oedema.[36] The dangers of overly aggressive volume replacement have been shown in adults and children. In the multicentre FEAST study of more than 3000 critically ill African children with sepsis and impaired perfusion, more than half (57%) of whom had malaria, those given fluid boluses had a higher mortality at 48 hours than those who were not (relative risk for bolus vs. control, 1·45; 95% CI, 1·13 to 1·86).[37] Severe anaemia (~Hb<50 g/L) requires urgent correction but evidence regarding optimal transfusion practices is lacking. A trial is ongoing to define this in children. [23, 38, 39] Seizures should be controlled with benzodiazepines; if recurrent, loading with longer-acting anticonvulsants should be considered with close monitoring for respiratory depression. Routine seizure prophylaxis with phenobarbital given to Kenyan children with cerebral malaria in a placebo-controlled trial was associated with increased rates of respiratory depression and death.[40] Other supportive measures depend on the clinical manifestations and the level of care available. Early renal replacement therapy is recommended. If there is no access to endotracheal intubation a nasogastric tube should be passed but enteral feeding delayed for 72 hours to reduce the risk of aspiration pneumonia.[41]

Patients with cerebral malaria should have blood cultures taken, a lumbar puncture performed and broad spectrum antibiotics commenced, pending negative culture results and clinical improvement. Severe malaria is associated with concomitant bacterial sepsis, with non-typhoidal *Salmonella* bacteraemia described frequently in African children.[42] Coinfections with other tropical diseases are unusual in travellers but more common in hyperendemic countries where a large proportion of the population carry malaria parasites, often without symptoms.

Patients with severe malaria should have frequent monitoring of vital signs, conscious level, glucose, renal function and haemoglobin. Monitoring parasite density (6-12 hourly) confirms parasite clearance (typically within 72 hours) but clinical improvement often takes considerably longer.[43, 44] Once the patient is conscious, able to eat and drink and has received at least 24 hours of parenteral therapy, oral follow on treatment with an artemisinin based combination treatment is advised.

Adjunctive therapies for severe malaria

Various adjunctive therapies have been evaluated without success in terms of improving the outcome from severe malaria, including steroids, anti-TNF, mannitol, N-acetylcysteine (antioxidant properties), exchange transfusion, and levamisole (inhibitor of sequestration).[45, 46 47]

Severe vivax and knowlesi malaria

Severe vivax malaria tends to occur in patients with co-morbidities in endemic countries and has not been observed frequently in returned travellers. Respiratory failure and acute kidney injury have

been reported repeatedly in fatal cases.[48, 49] One of the most common manifestations of severe vivax malaria in children is respiratory distress. [50] Coma is a rare occurrence in patients with vivax parasitaemia and no other apparent cause.[51] The main burden of serious morbidity and mortality from vivax malaria is secondary to severe anaemia, with young children particularly vulnerable. Rates of hospitalisation and death from vivax malaria approach those for falciparum malaria in some parts of the world such as Papua in Indonesia.[50, 52] Severe knowlesi malaria is associated with high parasite densities and similarly may present as acute kidney injury, shock or respiratory failure.[53]

Management of uncomplicated malaria

The main considerations when prescribing antimalarials are the infecting species and the risk of drug resistance. Comprehensive, evidence-based guidelines for the treatment of uncomplicated malaria are available on the website of the World Health Organization.[54] Given the global spread of *P. falciparum* resistant to chloroquine and antifols, artemisinin-based combination treatments (ACTs) are recommended for the treatment of falciparum malaria or falciparum mixed with non-falciparum species, except in the first trimester of pregnancy. ACTs consist of a combination of an artemisinin derivative which rapidly reduces parasitaemia and a partner drug which removes residual parasites over a longer period. These properties make them the drugs of choice to treat knowlesi malaria as well.[53] The leading ACTs in use are artemether-lumefantrine, artesunate-amodiaquine, dihydroartemisinin-piperaquine, artesunate-mefloquine and artesunate+sulfadoxine-pyrimethamine. The first four ACTs on this list exist as fixed-dose combinations, with paediatric formulations available. Artemether-lumefantrine should be given with milk or food containing fat to enhance lumefantrine absorption. The ACTs were highly efficacious against all *P. falciparum* until recently when numbers of treatment failures increased in parts of Southeast Asia (**Figure 3**).

Atovaquone-proguanil is an alternative non-artemisinin based treatment which is useful for individual patients (e.g. returned travellers without hyperparasitaemia, or in combination with artesunate plus primaquine for patients in endemic countries who have failed treatment with standard ACTs); however it is not recommended for widespread implementation in endemic countries, due to the propensity for rapid emergence of atovaquone resistance.[54] Quinine remains efficacious, although requires a long course, is poorly tolerated, particularly by children, and needs to be combined with a second agent such as doxycycline or clindamycin.

Uncomplicated vivax, malariae and ovale malaria are treated with chloroquine, unless chloroquine-resistant *Plasmodium vivax* is likely (Indonesia, Oceania) when an ACT is used (Panel 2).[55] This should be followed by primaquine to eradicate dormant hypnozoites.

The decision to admit a patient with uncomplicated malaria to hospital will depend on the setting and local guidelines. It is common practice to admit non-immune returned travellers for an initial period of observation until clinical improvement and a fall in parasitaemia are observed.

Management of uncomplicated malaria in pregnancy

Early detection of malaria in pregnancy is vital. Uncomplicated falciparum malaria in the first trimester is treated with a seven day course of quinine and clindamycin. Safety data of first trimester ACT use have been reviewed and are reassuring, and treatment guidelines will be reviewed in the near future. [56] After 12 weeks gestation, treatment is as for non-pregnant patients.[57] Vivax malaria in

pregnancy is treated with chloroquine unless resistance is suspected (when quinine should be given), but radical cure with primaquine is contraindicated as the glucose-6-phosphate-dehydrogenase (G6PD) status of the foetus cannot be ascertained. The pharmacokinetic properties of several antimalarial drugs are different in pregnancy with a tendency towards lower drug exposure.[58] This makes treatment failure more likely, especially in non-immune women.

Congenital malaria

The diagnosis of congenital malaria is easy to miss, especially if the mother is asymptomatic. Clinical presentation mimics neonatal sepsis. Parenteral treatment (artesunate or quinine) should be given for at least the first dose in congenital falciparum malaria. Follow-on treatment is with an ACT. Congenital vivax malaria can be treated with oral chloroquine unless the infant is very unwell when parenteral drugs should be used, or if chloroquine resistance is likely, when either an ACT or quinine should be given.

Adjunctive primaquine therapy

To reduce the risk of relapse from dormant hypnozoites in the liver a course of the 8-aminoquinoline primaquine is added to the treatment of vivax or ovale malaria.[59] Primaquine causes dose-dependent haemolytic anaemia in patients with G6PD deficiency, hence testing for this enzymopathy is recommended; however, worldwide, access to testing is poor. The standard primaquine treatment regimen is long (14 days) and as a result difficult to adhere to. Doubling the daily dose to 1 mg base/kg body weight and shortening the course to seven days was shown to result in an increased risk of clinically significant haemolysis in G6PD heterozygotes (9/17 (53%) patients experienced a >25% fractional haematocrit reduction compared with 2/16 (3%) G6PD heterozygotes taking the standard 0·5mg/kg dose for 14 days; p = 0·022). [60] Failure of primaquine to suppress relapses has been linked to reduced metabolism of the drug in individuals with polymorphisms in the cytochrome P-450 isoenzyme 2D6 (CYP2D6).[61] The longer-acting 8-aminoquinoline tafenoquine is in clinical development to replace primaquine for radical cure as a single dose treatment but the risk of haemolysis remains.[62]

Primaquine is the only drug able to kill mature (Stage V) gametocytes of *P. falciparum*. In endemic areas prescription of a single low (0·25 mg/kg) dose of primaquine with an ACT is recommended to reduce the risk of onward transmission. This dose is considered safe in G6PD deficiency.

Safety of the antimalarial drugs

Antimalarials may have serious side-effects and the frequency with which they occur varies between populations. Important examples include quinidine-induced cardiotoxicity, hypoglycaemia and hypotension following quinine, which may also cause QT-prolongation, although much less frequently than following quinidine, hepatotoxicity and cutaneous hypersensitivity reactions to sulfadoxine-pyrimethamine (Stevens Johnson syndrome in ~1/10000 recipients) and neuropsychiatric reactions to mefloquine (~1/200-1-2000 at treatment doses).[54, 63]

Treatment failure

Malaria treatments are not always curative.[64-67] Treatment failure usually presents as a recurrence of symptoms with detectable parasitaemia two to six weeks after an apparently successful treatment

and is not always due to drug resistance. Alternative explanations include high parasite densities (particularly in non-immune individuals), poor drug bioavailability, non-adherence to therapy, and falsified or substandard antimalarials. [68] Relapse of vivax malaria is common after an episode of falciparum in Southeast Asia (~30% cases). [69]

Artemisinin-resistant falciparum malaria in Southeast Asia

Like combination treatments in other areas of medicine, ACTs were introduced to prevent or delay development of resistance in a population over time. [70] After more than a decade of use in Southeast Asia artemisinin resistance was confirmed, manifest by a phenotype of delayed parasite clearance.[71-73] Use of artemisinin monotherapies was probably a contributing factor. The loss of the rapid parasiticidal effect of the artemisinin derivatives led predictably to worsening partner drug resistance (to mefloquine and piperaquine) and reduced efficacy of the corresponding ACTs. [74, 75] Artemether-lumefantrine efficacy was poor in Cambodia in the early 2000s and has not been re-assessed since then. Molecular markers for parasite resistance to many of the commonly used drugs have been discovered.[74-77] High rates of ACT failure have now been reported from Cambodia, Thailand and Viet Nam.[78-80] A newer ACT, artesunate-pyronaridine, may be approved for use in these countries in the near future. Triple artemisinin based combinations (dihydroartemisinin-piperaquine with mefloquine and artemether-lumefantrine with amodiaquine) are being evaluated in an attempt to bridge the gap until new medicines become available.[81]

New antimalarial drugs

Investment in antimalarial drug discovery and development, with the creation of product development partnerships in the early 2000s, have revitalised a near empty drug pipeline. Most of the drugs under development are blood schizontocides with a few exceptions. [82-88] There are efforts to develop alternative transmission-blocking drugs as an elimination tool.[89]

Complications of malaria

Delayed haemolytic anaemia can follow artemisinin treatment of travellers with falciparum malaria.[90] The key event appears to be pitting, a splenic process where ring-stage parasites killed by artesunate are expelled from their host erythrocytes which return to the circulation, but with a reduced lifespan (Figure 1).[91] This explains why the diagnostic antigen PfHRP2 persists for weeks after artemisinin treatment (it is exported to the erythrocyte periphery); indeed PfHRP2 concentration following parasite clearance predicts later haemolysis.[28] Treatment with quinine and other slowly acting drugs allows unhindered development of parasites until sequestration, so haemoglobin falls early. Delayed haemolysis can hence be considered a predictable event related to the early beneficial effect of artemisinins.[91] The problem appears to be somewhat less in African children[92] presumably because of developing immunity although cases have been documented. [93] It has also been suggested that post-artesunate delayed haemolysis could contribute to the high frequency of haemoglobinuria reported in eastern Uganda recently.[94]

Other haematological complications of malaria include hyperreactive malarial splenomegaly (HMS), and rarely, splenic rupture. HMS is characterised by massive splenomegaly, raised IgM antimalarial antibodies and anaemia. It responds to a prolonged course of weekly treatment with antimalarial drugs at prophylactic doses.[95]

Neurological complications following an episode of severe malaria range from a reversible post-malaria neurological syndrome to permanent deficits including visual, motor or language disorders and epilepsy. In a study of African children with severe falciparum malaria the incidence of persistent neurological sequelae in the 4898 children who survived was 3·2%.[33] *Plasmodium malariae* may be complicated by anaemia and the nephrotic syndrome.[96]

Malaria co-infection with HIV

HIV coinfection increases malaria severity and parasite densities tend to be higher. Cotrimoxazole prophylaxis is protective against malaria and concomitant antifolate antimalarial drugs should be avoided. [97] There are drug-drug interactions between common antimalarials and antiretroviral drugs, e.g. efavirenz increases amodiaquine exposure and the risk of toxicity so these drugs should not be coadministered. Efavirenz decreases lumefantrine exposure but no formal recommendations have been made to adjust the dose.[98, 99]

Immunity

Repeated exposure to malaria leads over a long period to premunition - protection from disease but ongoing blood stage infection. In *P. falciparum* this involves sequential acquisition of antibodies to PfEMP1 subtypes. Asymptomatic falciparum or vivax parasitaemia are common in areas of high endemicity and use of more sensitive molecular detection methods for malaria has revealed that parasites may persist for years longer than thought possible previously.[100] Antibodies act on parasite blood stages to inhibit replication with complement fixation thought to play a role.[101] Antigen presentation takes place at different stages of the parasite life-cycle and T cells regulate both liver and blood stages.[102] Immunity is lost steadily after an individual leaves an endemic area, or in a population with falling transmission.

Predisposing and protective factors

A wide range of inherited and acquired factors influence an individual's chance of infection and severe illness with malaria (Panel 3). Haldane's hypothesis suggesting that inherited human red cell conditions reflect evolutionary protection against malaria continues to be confirmed and refined.[103] Powerful studies combining clinical data from thousands of patients and controls with advanced sequencing methods have confirmed the protective role of structural variants of β-globin against severe malaria, the HbS variant (in heterozygous form causing sickle cell trait) protecting ten-fold, and the west African HbC variant to a lesser extent.[104] A consensus on the mechanism of protection has still to emerge although suppression of parasite multiplication is probably involved given protection against both cerebral malaria and severe anaemia.

The protective effect of X-linked G6PD deficiency, the commonest inherited human enzymopathy, against *P. falciparum* appears complex. The A- allele is common in many parts of Africa, and male hemizygotes and female heterozygotes have reduced risk of cerebral malaria, but male hemizygotes and female homozygotes have increased risk of severe malarial anaemia. [104] In Asia, G6PD deficiency protects against vivax malaria.[105, 106]

Genetic differences in red cell surface proteins also influence malaria. It has long been known that individuals negative for the Duffy antigen receptor for chemokines (DARC) are resistant to *P. vivax*

and *P. knowlesi*, which preferentially invade reticulocytes via their Duffy binding protein. This protection has turned out not to be absolute; reports of vivax malaria in Duffy negative individuals indicate that alternative ligands can mediate invasion.[107, 108] Nevertheless clinical vivax malaria remains rare in countries where the population is completely Duffy negative (most of sub-Saharan Africa).

Other families of red cell surface proteins also influence malaria infection, with individuals with blood group O relatively protected against severe falciparum malaria and those with group B at higher risk.[104] Recently a complex rearrangement in the polymorphic MNS blood group system (the 'DUP4' haplotype), producing hybrid glycophorin proteins, was shown to be associated with protection against severe malaria in East Africa.[109]

Prevention of malaria

Malaria is prevented by chemoprophylaxis, vaccination, bite-avoidance and vector control measures (Panel 4).[110]

Chemoprophylaxis

Target groups for chemoprophylaxis are pregnant women, young children and travellers. Intermittent preventive therapy in pregnancy (IPTp) and infants (IPTi) have been slow to be scaled up in many areas and their impact is threatened by SP resistance.[111 112] The use of alternative antimalarials such as dihydroartemisinin-piperaquine is under evaluation. Intermittent screening and treatment of pregnant women (ISTp) has not been demonstrated to be an effective alternative strategy to IPTp, attributed to lack of sensitivity of current RDTs to detect malaria in pregnancy.[112, 113] Seasonal malaria chemoprevention in children with SP+amodiaquine has been adopted widely and reduced malaria in areas of highly seasonal transmission in the Sahel.[114]

To prevent malaria in travellers, the choice of chemoprophylaxis should take into account the risks of malaria and drug resistance, which should be balanced with the risk of drug toxicity. Providing stand-by emergency treatment packs may be a more suitable option in some cases. [115] Atovaquone-proguanil and doxycycline are prescribed as prophylaxis frequently. Weekly mefloquine at prophylactic doses is a convenient option but unpopular due to concerns about neurotoxicity. Primaquine is a highly effective causal prophylactic agent with the added advantage of enhanced protection against vivax malaria (hypnozoites stages) but requires exclusion of G6PD deficiency. [116]

Vaccine development

Malaria subunit vaccines are designed to provide immunity against proteins exposed at critical stages of the lifecycle. Targeting sporozoite stages via one of the surface proteins that mediate homing to the liver and host cell traversal / invasion aims to reduce frequency of infection. The RTS,S/AS01 vaccine based on *P. falciparum* circumsporozoite protein is the most studied vaccine. In a landmark study in African children, RTS,S/AS01 provided significant protection against falciparum malaria infection over a 3-4 year period; in older children vaccine efficacy was 36·3% (95% CI 31·8–40·5) with a 20-month booster and 28.3% (23·3–32·9) without.[117] However efficacy was relatively lower (25·9% (19·9–31·5) with booster and 18·3% (11·7–24·4) without) in very young children (6-12 weeks old at first dose). Further, in contrast to earlier data, RTS,S/AS01 did not provide even efficacy across all strains.[118] Longer term follow-up at one centre revealed a higher incidence of malaria in

later years in vaccinated children with higher-than-average exposure to malaria.[119] An overall reduction in long-term mortality remains to be demonstrated. The WHO is supporting pilot implementation of the four-dose regimen in 5-to 17-month old children in three countries, allowing study of long-term outcomes, safety and practicality.

A contrasting approach to producing sporozoite-based immunity is the *Plasmodium falciparum* sporozoite (PfSPZ) vaccine, an intravenous injection of irradiation-attenuated sporozoites. PfSPZ has now entered clinical trials in Africa;[120] again the challenges are likely to centre on obtaining durable protection against all relevant strains.

Use of merozoite-stage proteins as vaccine targets aims to reduce asexual replication rate and hence protect against disease rather than produce sterile immunity, potentially allowing immunity to develop naturally while the vaccinee is protected from severe disease. Of the various extracellular merozoite proteins that collectively mediate erythrocyte invasion by *P. falciparum*,[121] there is considerable interest in targeting PfRh5 since its binding (to the Ok blood group antigen basigin) is critical for erythrocyte invasion.[122] Another asexual stage vaccine targets the product of the PfEMP1 VAR2CSA type aiming to prevent parasitized cell binding to CSA1 and hence protect against placental malaria.[123]

Transmission-blocking vaccines against sexual-stage antigens aim to generate antibodies that are ingested in the mosquito blood meal, potentially providing immunity at a population level. There is also increasing study of how vaccine responses to multiple targets might synergise to produce higher levels of overall protection,[124] although such an approach is likely to increase costs substantially. Vaccine development as a whole is complicated by the absence of reliable laboratory correlates of immunity.

Vector control

More than forty species of *Anopheles* are important malaria vectors. The mainstays of vector control are long-lasting insecticide (pyrethroid) treated bed nets (LLINs) and indoor residual spraying with insecticides. LLINs have reduced morbidity and mortality from malaria and have the biggest impact in high transmission areas where vectors bite indoors at night, such as the highly successful *Anopheles gambiae* complex.[110] Their success is threatened by widespread pyrethroid resistance in Anopheline vectors, although the relationship between resistance and LLIN efficacy is not well-characterised.[125-127] Alternative insecticides are needed urgently. In the meantime addition of the synergist piperonyl butoxide to pyrethroid-treated bed nets has being evaluated in some countries with mixed results. Widespread deployment has not yet been recommended by WHO.[121] Bite-prevention strategies including topical repellents have not been shown to have an impact on malaria incidence.[128, 129] Other approaches under consideration are mass treatment with ivermectin, which shortens mosquito survival, and transgenic mosquitoes.[130-133]

From control to elimination

Progress towards malaria elimination is uneven. Indigenous cases in Europe, central Asia (north of Afghanistan), Sri Lanka and several countries in Latin America are now extremely rare. However, in many sub-Saharan African countries, where transmission is highest, eliminating malaria has proved more difficult and there are signs that progress in this direction has stalled.[1, 6, 134] Areas with civil

disruption have experienced significant increases in malaria, exemplified by Venezuela. Pilot studies of mass drug administration (MDA) of ACT with single-dose primaquine to accelerate elimination of drug-resistant malaria in Southeast Asia have taken place and early reports suggest it is effective and safe.[135]

Controversies and uncertainties

There is more to be learned about the pathogenesis of severe vivax malaria, the relationship between pyrethroid resistance and LLIN efficacy, and the longer term impacts of MDA.[129] Elimination of vivax malaria will require increased uptake of primaquine which means addressing safety concerns in populations where G6PD deficiency is common. Elimination of human knowlesi malaria infections while there is an ongoing reservoir of parasites in macaques is another challenge. Whether artemisinin resistance spreads or pops up in different locations has been the subject of debate; recent evidence suggests both are true.[136] The impact of artemisinin resistance on artesunate efficacy in the treatment of severe falciparum malaria is unknown because severe malaria is now rare in Southeast Asia. The loss of ring stage susceptibility to artemisinin raises the possibility that the treatment advantage over quinine has been eroded. Addition of parenteral quinine to artesunate has been proposed to provide a safety net for patients with presumed artemisinin resistance but there is no evidence to support this practice currently.

Future perspectives

More than 130 years have passed since the protozoan cause of malaria was discovered. Over this period there have been many scientific breakthroughs, with a subset translating into interventions capable of reducing the burden of disease and death, notably the discovery and development of antimalarial drugs and insecticides.

The progress towards elimination in some countries shows that current tools can be enough to eliminate malaria if the right conditions are in place: political commitment, access to healthcare, and adequate human and financial resources. There is evidence that access to high quality ACTs is still much too low (<25%) in some areas. [137] The spread of pyrethroid resistance among *Anopheles* vectors and increasing reports of ACT failures in Southeast Asia signal that the window of opportunity to eliminate malaria with current tools may be closing. Increased resources for disease control usually only come in times of crisis, but a concerted effort now could capitalise on recent gains and accelerate progress towards elimination.

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Authors' Contributions

EAA performed the literature search. EAA and CW wrote the first draft of text. APP drafted the tables and panels and Figure 3. All authors reviewed and approved the final version of the manuscript.

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Figure titles and legends

FIGURE 1: Human stages of the malaria lifecycle. The inset illustrates the differing outcomes of treatment of falciparum malaria with quinine and artesunate. Quinine kills sequestered parasites but not circulating rings, so in non-immune patients treated with quinine, sequestration is the dominant method of parasite clearance. Artesunate brings the advantage of rapid action against both circulating rings and sequestered parasites; killing of early stages produces pyknotic parasite forms which the spleen removes by pitting, leading to potentially large numbers of once-infected red cells with reduced lifespans and the phenomenon of late haemolysis. These cells contain the PfHRP2 antigen (indicated by green outline) explaining why despite parasite clearance this persists (potentially allowing prediction of late haemolysis). Individuals with partial immunity to malaria clear both parasitized and once-infected red cells by antibody-mediated phagocytosis in the spleen relatively rapidly.

FIGURE 2: Blood films showing microscopic appearances of the human malarias. All parasite stages are visible in peripheral blood except *P.falciparum* RBCs containing mature trophozoites where the vast majority are sequestered in deep vessels. Thin blood films were prepared from specimens taken from patients with clinical malaria, stained with modified Field's stain and examined by light microscopy under oil immersion at x1000 magnification.

FIGURE 3: Global distribution of drug-resistant *P. falciparum*. Countries are shown according to the level of resistance of local *P. falciparum*; the thirteen countries approaching elimination (as defined in the World Malaria Report 2016) are also shown, including Tajikistan which has had no cases since 2014. The inset graphs show WHO estimates of global annual malaria case numbers and deaths from 2000 to 2015. [138]

PANELS

Search Strategy panel

Search strategy and selection criteria

We searched PubMed, Embase and the Cochrane Library for all clinical trials, meta-analyses, systematic reviews, and diagnostic test accuracy studies published between 2014 and July 2017, using the search term "malaria". International malaria treatment guidelines and policy documents on the website of the World Health Organization were also consulted. References cited in these publications were screened to identify other recent original journal articles and highly relevant older references e.g. definitive trial reports, or articles linked to a particular discovery

Panel 1 Diagnostic criteria for severe malaria [22]

Rapid assessment
• Prostration • Confusion/agitation (with GCS>11) • Coma (Glasgow Coma Scale <11 or Blantyre Coma Scale <3 in children) • Respiratory distress (acidotic breathing) • Convulsions • Shock: Prolonged capillary refill time (> 2 seconds), +/- systolic blood pressure BP<80mmHg in adult (<70 in children) • Pulmonary oedema (radiological) • Abnormal bleeding • Jaundice • Anuria
Full criteria (addition to rapid assessment)
• Haemoglobin <7g/dL in adult <5 g/dL in children • Haemoglobinuria • Hypoglycaemia (blood glucose <2·2 mM or <40 mg/dl) Blood chemistry • Acidosis (i.e. base deficit >8 meq/l or plasma bicarbonate <15 mM or venous plasma lactate >5 mM) • Acute kidney injury (creatinine 3 mg/dl or urea >20 mM) Parasitaemia • Very high asexual parasitaemia (≥ 10% of infected RBCs) Note: National guidelines may vary e.g. UK parasitaemia cut-off is 2%[139]

Panel 2 Drug treatment of malaria[54]

SEVERE MALARIA
Initial treatment -Intravenous artesunate (2·4 mg/kg per dose hours 0, 12 and 24; then 24-hourly. In patients with body weight <25 kg unit dose is 3·0 mg/kg) Alternatives -Intravenous quinine infusion; loading dose of 20 mg/kg (given over 4 hours) at admission then 10 mg/kg (given over 2 hours) every 8 hours. -Intramuscular artemether injection: 3·2mg/kg initial dose, then 1·6mg/kg every 24 hours *Once able to eat and drink* Oral treatment with an ACT for 3 days (NOT mefloquine due to increased rick of post-malaria neurological syndrome)
UNCOMPLICATED MALARIA
Uncomplicated Plasmodium falciparum[a] *or P.knowlesi malaria* -Artemether-lumefantrine 1·4–4 mg/kg of artemether and 10–16 mg/kg of lumefantrine twice daily for 3 days with food containing fat, or -Dihydroartemisinin-piperaquine 4 mg/kg of dihydroartemisinin and 18 mg/kg of piperaquine once daily for 3 days (children with body weight <25 kg should receive at least 2·5mg/kg/day of dihydroartemisinin and 20 mg/kg/day piperaquine), or -Artesunate 4mg/kg/day with mefloquine 8mg/kg/day for 3 days, or -Artesunate 4mg/kg/day with amodiaquine 10mg base/kg/day for 3 days, or -Artesunate 4mg/kg/day for 3 days with single dose sulfadoxine-pyrimethamine (25mg/kg-1·25mg/kg) *Chloroquine-sensitive P.vivax,*[b] *P.ovale, P.malariae* [c] -Chloroquine 10mg base/kg/day at hour 0 and hour 24 followed by 5mg base/kg at hour 48

Target doses are shown in the table. See supplementary appendix for full dosing information of different formulations and non-artemisinin-based treatment options

[a] Choice of ACT to treat *P.falciparum* depends on local resistance patterns. Mefloquine is contraindicated in patients with a history of epilepsy of neuropsychiatric disorders

[b] Chloroquine-resistant *P.vivax* is treated with one of the ACTS (except artesunate+ sulfadoxine-pyrimethamine)

[c] Initial treatment of *P.vivax* or *P.ovale* should be followed by a course of primaquine to prevent relapse, if no contraindications (G6PD deficiency, pregnancy, age < 6 months)

Panel 3 Factors predisposing to and protecting from severe malaria

Predisposing Factors	**Protective Factors**
Genetic factors • Sickle cell disease • Blood Group B Acquired factors • Pregnancy and early postpartum period • Malnutrition • HIV • Hyposplenism	Genetic factors • Haemoglobin AS (sickle cell trait) • Haemoglobin C • Thalassaemia • Glycophorins A and B • Blood Group O

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Chemoprophylaxis

- Intermittent preventive treatment (IPT)
    - Pregnant women (IPTp) receive sulfadoxine-pyrimethamine (SP) at all antenatal care visits from 2nd trimester (minimum dose interval of one month)
    - Infants in moderate transmission areas receive SP with routine immunisations (IPTi)
- Seasonal malaria chemoprevention (SMC)
    - children aged 3-59 months in areas of seasonal transmission in the Sahel (intermittent SP+amodiaquine at treatment doses, maximum four courses)
- Fixed-term
    - Travellers (atovaquone-proguanil, doxycycline, mefloquine or primaquine)
    - HIV-infected patients receiving cotrimoxazole as secondary prophylaxis for opportunistic infections are protected against malaria

Vaccination

- RTS,S/AS01 in children (4 doses for children 5-17 months) is the only registered malaria vaccine (still under evaluation)

Vector control/bite-prevention

- Long-lasting insecticide treated bednets (LLINs): most effective in high-transmission areas where vectors rest indoors at night
- Indoor residual spraying (with insecticides)
- Repellents (topical and spatial): convincing protective effect not shown

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