Malaria review

Book · September 2019

CITATIONS
0

READS
86

1 author:

Mosab Nouraldein Mohammed Hamad
Elsheikh Abdallah Elbadri University
560 PUBLICATIONS **167** CITATIONS

SEE PROFILE

Malaria is a major economical and health problem in many regions around the globe . 40% of world population at risk of malaria, pregnant ladies and children under 5 years old are mostly affected . In this book we discussed about history, epidemiology, immunity, diagnosis, treatment of malaria. Up to date there is no commercial vaccine available to this fatal disease ,despite to great effort excreted by charity organizations and global foundations.

Madiha Elfadil Elkhairi , BSC,MSC , Medical Parasitology. Mosab Nouraldein Mohammed Hamad, BSC,MSC, Medical Parasitology. Tarig Mohamed Elfaki, BSC,MSC, Medical Parasitology.

Madiha Elfadil Elkhairi
Mosab Nouraldein Mohammed Hamad
Tarig Mohammed Elfaki

Malaria review

978-620-0-30451-3

LAP LAMBERT Academic Publishing

Madiha Elfadil Elkhairi
Mosab Nouraldein Mohammed Hamad
Tarig Mohammed Elfaki

Malaria review

Madiha Elfadil Elkhairi
Mosab Nouraldein Mohammed Hamad
Tarig Mohammed Elfaki

Malaria review

LAP LAMBERT Academic Publishing

Imprint

Cover image: www.ingimage.com

Publisher:
LAP LAMBERT Academic Publishing
is a trademark of
International Book Market Service Ltd., member of OmniScriptum Publishing Group
17 Meldrum Street, Beau Bassin 71504, Mauritius

Printed at: see last page
ISBN: 978-620-0-30451-3

Malaria Review

Madiha Elfadil Elkhairi

Mosab Nouraldein Mohammed Hamad

Tarig Mohamed Elfaki

List of contents:

Dedication

{To our families and our best friends}

Acknowledgement

We are deeply grateful to our teachers, colleagues for their support and encouragement.

Introduction

Malaria history:

Malaria remains one of the main killers of humans' universal, threatening the lives of more than 1/3 of the world's population [1]. It is an prehistoric disease and references to what was almost certainly malaria occur in a Chinese document from about 2700 BC, clay tablets from Mesopotamia from 2000 BC, Egyptian papyri from 1570 BC and Hindu texts as far back as the sixth century BC. Such historical records must be regarded with caution but moving into later centuries we are beginning to step onto firmer ground. The early Greeks, including Homer in about 850 BC, Empedocles of Agrigentum in about 550 BC and Hippocrates in about 400 BC, were well aware of the characteristic poor health, malarial fevers and enlarged spleens seen in people living in marshy places. For over 2500 years the idea that malaria fevers were caused by miasmas rising from swamps persisted and it is widely held that the word malaria comes from the Italian mal'aria meaning spoiled air although this has been disputed. With the discovery of bacteria by Antoni van Leeuwenhoek in 1676, and the incrimination of microorganisms as causes of infectious diseases and the development of the germ theory of infection by

Louis Pasteur and Robert Koch in 1878-1879, the search for the cause of malaria intensified. Scientific studies only became possible after the discovery of the parasites themselves by Charles Louis Alphonse Laveran in 1880 and the incrimination of mosquitoes as the vectors, first for avian malaria by Ronald Ross in 1897 and then for human malaria by the Italian scientists Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava between 1898 and 1900. Excellent histories of this disease include those by Celli [2], , Stephens [3], Scott [4], Russell [5], Foster [6], Garnham [7,8], Harrison [9], Bruce-Chwatt [10], Desowitz [11], McGregor [12], Poser & Bruyn [13] and Schlagenhauf [14]. . It is believed that its homeland is West Africa malaria (P. falciparum) and Central Africa (P. vivax). Four species of malaria parasite infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* [15].

Malaria probably originated in Africa and accompanied human migration to the Mediterranean shores, India and South East Asia. In the past it used to be common in the marshy areas around Rome. As malaria is a disease mostly of tropical and subtropical areas, it is particularly prevalent in sub-Saharan Africa, but also common throughout other tropical regions of China, India, Southeast Asia, South and Central America [16, 17]. In Nigeria, before independence, the colonialists established Government Reservation Areas (GRA) in an attempt to build their homes far away from the natives as it was found that the travelling/flying distance of these mosquitoes from the breeding grounds was a limiting factor in spreading the parasites. Nigeria's quest for

effective control of malaria began well before the WHO global malaria eradication period between 1955 and 1968 [18].

Malaria epidemiology

Malaria is one of the most important public health problems in term of morbidity and mortality, causing more than 200 million cases and 655.000 deaths every year [19]. According to the World Health Organization (WHO) Malaria Report 2011, a total of 106 countries in the world are at risk of transmission of malaria infection .A total of 216 million estimated malaria cases occurred in 2010, 81% of which were reported in the African Region, followed by South East Asia (13%) and Eastern Mediterranean Region (5%). The total number of malaria deaths was estimated to be 655.000 in 2010; 91% of whom occurred in the African Region, 6% in South-East Asia and 3% in Eastern Mediterranean Region .Although the proportion of people exposed to malaria parasites has decreased during the last century, the absolute number of people at risk for malaria infection increased from 0.8 billion in 1900 to 3.3 billion in 2010, as a consequence of the absolute increase of the population living in malaria-endemic regions [19,20].

However, between 2005 and 2010 malaria cases decreased from 244 million to 216 million; moreover, malaria mortality rates showed a global reduction of 26% between 2000 and 2010.

Malaria in humans is caused by 5 *Plasmodium* parasites: *Plasmodium falciparum*, *P. vivax*, *P. malariae, P. ovale* and *P. knowlesi*. The current distribution of human-pathogenic *Plasmodium* species shows preponderance of *P. falciparum* in tropical Africa, while *P. vivax* prevails over *P. falciparum* in South America. Both *P. falciparum* and *P. vivax* are prevalent in south-eastern Asia and western Pacific. Although *P. malariae* may occur in all malarious areas, its prevalence is generally low. In tropical Africa, *P. falciparum* and *P. malariae* co-infection is sometimes encountered. *P. ovale* is widespread principally in tropical Africa whereas *P. knowlesi* infection occurs only in certain forested areas of South-East Asia.

Malaria burden is hard to estimate, particularly in low income countries where data collection and reporting quality is poor. Incomplete and discontinuous reports from single health facilities may alter final global malaria prevalence. Malaria cases are often under-diagnosed in hyper endemic countries, where mild symptoms of chronic malaria my possibly lead to misdiagnosis. On the contrary, over-diagnosis may also occur. In fact, not all reported malaria cases are confirmed by microscopy or others assay, such as rapid diagnostic tests (RDTs). Furthermore, in hyper endemic areas febrile illnesses from different causes might be misdiagnosed with malaria [21]. Anyway, WHO guidelines recommend that microscopy or RDTs should be used to confirm all malaria cases.

Another issue is the lack of population denominator that makes the real incidence of malaria difficult to assess. Data emerging from WHO reports just estimate malaria incidence and mortality, reporting malarial cases and malarial death from the different WHO regions, collected by Ministries of Health of different countries. These data do not reflect the real incidence in the general population. Nevertheless, they are good indicators to assess malarial control programmes and to estimate the impact of malaria infection in health systems.

Malariometry:

The term *endemicity* is a proxy to indicate disease prevalence. Areas presenting the same level of endemicity often have similar characteristics of disease distribution, guiding malaria experts to design, implement and monitor control and prevention activities [22].

Malaria endemicity is a very complex issue, that is influenced by factors related to the man-host interactions (agricultural activities, nocturnal activities, migration movements, wars, limited resources), to the parasite (different species, sporogonic cycle length, drug susceptibility), to the vector (density, larvae breeding sites, temperature, receptivity, feeding pattern, longevity, insecticide susceptibility) and to the environment (physical – biological – socio-economic). Moreover, malaria incidence may fluctuate according to seasonality.

Different methods to classify malaria endemicity in a population exist. These methods includes (i) proportion of individuals in a population with a palpable enlargement of spleen (*spleen rate* [SR]), (ii) proportion of individuals in a population with a laboratory-confirmed parasite infection (*parasite rate* [PR]), (iii) number of infective bites per person (*entomological inoculation rate* [EIR]) and (iv) number of microscopically confirmed malaria cases detected during one year per unit population (*annual parasite incidence* [API]) [23].

Proportion of individuals with splenomegaly (SR) in a given population was the first method used to assess malaria endemicity during a malariometric survey in 1848 in India, where spleen dimension was assessed in selected population age groups. Thus, malariometry attention was focused on clinical manifestations of malaria. On the basis of splenomegaly prevalence rates in children from 2 to 9 years old, 4 different endemicity areas can be distinguished: *holo-endemic areas*, where proportion of people with splenomegaly is above 75%; *hyper-endemic areas*, where splenomegaly prevalence is between 51 and 75%; *meso-endemic areas*, with prevalence between 50 and 11%; *hypo-endemic areas*, where prevalence is below 11% [23].

Parasite rate (PR) assesses the proportion of individuals with microscopically confirmed presence of asexual parasites in peripheral blood. It's a technique that requires expert laboratory technicians and suffers of malaria seasonal variation.

Spleen and parasite rate are actually less used, whereas entomological inoculation rate (EIR) and annual parasite incidence (API) are utilized to prepare epidemiologic malaria maps that show malaria distribution in the world. Where data are unavailable, a model is required to predict malaria endemicity [24-28]. Many recent studies investigated a predictive framework known as model-based geostatistics (MBG) to asses' malaria endemicity [29-33].

In 2007, the world was stratified into three spatial representation: (i) areas without *P. falciparum* malaria risk, (ii) unstable risk areas (*P. falciparum* annual parasite incidence [PfAPI]: < 0.1 per 1.000 people per annum [pa]) and (iii) stable risk areas (PfAPI ≥ 0.1 per 1.000 people pa) [34]. The global area at risk of stable *P. falciparum* malaria was quantified in 29.7 million km^2, distributed into Africa (18.2 million km^2, 61.1%), Americas (6.0 million km^2, 20.3%) and

Central and South East Asia regions (5.5 million km^2, 18.6%). Of the 2.37 billion people exposed to *P. falciparum* transmission worldwide, 0.98 billion live in unstable risk areas [35, 36], whereas 1.383 billion live in stable risk areas, distributed into Africa (0.657 billion, 47.5%), Americas (0.041 billion, 2.9%) and Central and South East Asia (0.686 billion, 49.6%). Children are the most represented category, accounting for 32% of the population at risk in Americas and in Central and South East Asia. In Africa this percentage rises up to 43%.

P. vivax is transmitted in 95 tropical, subtropical and temperate countries [37]. People living at risk of *P. vivax* malaria infection are 2.85 billion, 91% living in Central and South East Asia region, 5.5% in America and 3,4% in Africa. As many as 57.1% of people exposed to *P. vivax* infection lives in unstable malaria areas.

Stable - unstable classification is another way to determine malaria endemicity. Macdonald defined malaria stability on the ground of the number of mosquitoes lifetime bites in the human host [38]. This vector-based index differentiated stable and unstable malaria. Vector-based classification is less used because of entomological-based metrics complexity, ethical concerns related to exposing human beings to malaria infection and measurement error issues [23].

Endemic regions Distribution:

Different malaria endemic areas have different epidemiological situations and also the feasible targets may differ. According to WHO, the following terminology should be adopted when referring to malaria endemic status: *Malaria control*: reducing the malaria disease burden to a level at which it is no longer a public health problem.

Malaria elimination: the interruption of local mosquito-borne malaria transmission; reduction to zero of the incidence of infection caused by human malaria parasites in a defined geographical area as a result of deliberate efforts; continued measures to prevent reestablishment of transmission are required.

Malaria eradication: permanent reduction to zero of the worldwide incidence of infection caused by a particular malaria parasite species. Intervention measures are no longer needed once eradication has been achieved.

Trends in malarial cases and deaths reflect control programmes, such as distribution of insecticide-treated nets (ITN), long lasting insecticidal nets (LLIN), and use of indoor residual spraying (IRS) and artemisinin combination treatment (ACT).

On the ground of slide positivity rate (SPR) and of the population at risk of malaria, WHO distinguishes areas with advance malaria control activities in (I) *pre-elimination phase*, (II) *elimination phase*, (III) *prevention of reintroduction* and (IV) *malaria-free stages*.

Most malaria cases and deaths occur in the *African Region*. As a consequence of implementation programs, high burden countries of African Region, such as Madagascar, Sao Tome and Principe, Eritrea, Rwanda and Zambia, showed a decrease in malaria cases up to 50% between 2000 and 2009 [39].

Rwanda showed a decrease by 74% of confirmed malarial cases between 2005 and 2010 and slide positivity rate decreased from 35% to 9%. Moreover, number of malaria hospital admissions and malaria deaths showed a decrease of 65% and 55% respectively. Zanzibar, belonging to United Republic of Tanzania, showed a dramatic decrease of malaria admissions and deaths due not only to the efficacy of control strategies, but also to favorable geographic position. In low-transmission countries of African Region control strategies have also been performed [19].

In 15 countries of the WHO *Region of the Americas*, where *P. vivax* is the most represented species, reductions of more than 50% in the number of the reported cases were observed. During 2010, malaria transmission occurred in 21 countries, of which 17 are in the control stage and 4 are in the pre-elimination stage. Bahamas and Jamaica are in the prevention of reintroduction phase. In Ecuador, malaria cases dropped from 105.000 in 2000 to 4.120 in 2009, a reduction of 96% due to IRS, LLINs distribution, strengthening of malaria diagnosis and treatment and also due to Global Found, UNICEF, USAID and government funds invested in malaria control [19].

In 2010, 2.4 million confirmed malaria cases were reported in WHO *South-East Asia Region*. India accounts for 66% of confirmed cases, even though a reduction of 28% of the cases between 2000 and 2010 was observed. In 2010, malaria deaths were 2.426 as reported from eight countries of the region, most of all reported in India. Democratic People's Republic of Korea and Sri Lanka are actually in pre-elimination phase. Bangladesh, Bhutan, the Democratic Republic of Timor-Leste, India, Indonesia, Myanmar, Nepal and Thailand are in the control phase.

In the WHO *European Region*, the number of autochthonous cases decreased from 32.394 in 2000 to 176 in 2010. All malaria cases are now attributable to *P. vivax* infection; no *P. falciparum* cases occurred since 2008. Malaria cases were identified in Azerbaijan, Kyrgyzstan, Tajikistan, Turkey and Uzbekistan. Georgia reported no cases in 2010 and Turkmenistan was declared malaria-free in October 2010. A particular case is represented by Greece, a country that was declared malaria-free from 1974.

Since June 2011 a total of 63 autochthonous malaria cases have been reported [39], all due to *P. vivax* infection. Cases occurred mostly in the southern region of the country, specifically of the Evrotas delta area of Laconia district in agricultural area with large migrant populations [39, 40], Other cases occurred in the Evia/Euboea (island east of the Central Greece region), Eastern Attiki, Voitia and Larissa districts [39].

In the WHO *Eastern Mediterranean Region*, Islamic Republic of Iran and Saudi Arabia are in the elimination phase, while Egypt, Iraq, Oman and Syrian Arab Republic are in prevention of reintroduction phase. Morocco was confirmed malaria-free in May 2010. Afghanistan, Djibouti, Pakistan, Somalia, Sudan, South Sudan and Yemen are in the control stage, and they still represent high malaria transmission areas.

As many as 262.000 confirmed cases were reported from the WHO *Western Pacific Region* in 2010. Papua New Guinea, Cambodia and Solomon Island account for 70% of these malarial cases. China, Philippines, Republic of Korea and Vietnam showed a decrease in malaria cases up

to 50% between 2000 and 2010, while other countries showed a more slowly decrease (e.g. Cambodia, Lao People's Democratic Republic, Malaysia, Solomon Island, Vanuatu).

Plasmodium Species Distribution:

Plasmodium species are differently distributed in the world. Prevalence of malaria cases and deaths differs during different seasons, as mentioned before. Prevalence data must be related to season and to endemicity of each country.

1. ***Plasmodium falciparum*:**

   P. falciparum is widespread in nearly all malaria endemic countries. A study identified 2.37 billion people at risk of *P. falciparum* transmission worldwide, 26% located in the African Region and 62% in South East Asian and Western Pacific regions [35]. In Africa, many epidemiological studies suggest that *P. falciparum* is the most prevalent malarial species. Blood samples were collected between 1998 and 2006 from nine different African countries and analyzed by PCR for the presence of each of the four human malaria parasites [41]. Out of 2.588 samples, 1.737 were positive for *Plasmodium* species and 1.711 (98,5%) were positive for *P. falciparum* considering both mono and mixed infection. Another study performed in 4 villages in Mulanda sub-county, in eastern Uganda, showed a prevalence of *P. falciparum* infection of 94% during rainy season, from July to December, using thin film diagnosis [42]. A study performed in metropolitan Lagos, Nigeria, showed a microscopic prevalence of *P. falciparum* species of 88, 5% in pregnant women attending antenatal care clinic, during one observation year [43]. In Asia, *P. falciparum* and *P. vivax* are the two prevalent species. In India, *P. falciparum* is mostly widespread in Orissa state [44, 45] while in the west of the country mixed infections are predominant [44]. In Bangladesh, samples collected during 3 years from febrile patients and analyzed by species-specific PCR showed a *P. falciparum* prevalence of 81, 5%. [46]. In Cambodia, *falciparum* prevalence among residents of 8 villages was about 59% using a new PCR technique [47]. In Thailand (Tak, Chantaburi, Prachuab Khirikhan, Yala and Narathiwat Provinces), PCR research of *P. falciparum* among febrile patients from October 2006 to September 2007 showed a prevalence of 43, 5% both in mono and in mixed infection [48]. Samples collected from 146 selected patients with uncomplicated malaria in 2008 in southern Myanmar underwent PCR analysis to investigate malaria parasites: the prevalence of *P. falciparum* was 52,1% considering mono and mixed infection [49]. In South America *P. vivax* is the predominant species, followed by *P. falciparum* (25.7%) [50]. Most of the malaria cases occur in Brazil; the others are distributed in 20 other countries of Central and South America [50].

2. Plasmodium vivax:

In Central and Western Africa *P. vivax* infection is rare because of the high prevalence of the red blood cells Duffy negative phenotype in the population, interfering with *P. vivax* merozoite entry into the red blood cells. A large study carried out in nine African countries failed to detect *P. vivax* species [41]. However, there are some evidence of *P. vivax* transmission in West and

Central Africa. In Congo, specific *P. vivax* antibodies were researched in 409 samples from patients coming from a health center located on the west coast, where Duffy antigen is expected to be > 95%. Out of the 409 samples, 55 (13%) tested positive for specific *P. vivax* antibodies [51]. study from Kenya demonstrated the presence of *P. vivax* among mosquitoes; in addition, *P. vivax* DNA was amplified and sequenced in blood of two Duffy negative children [52]. In eastern and southern Africa only 5% of malaria infections are attributable to *P. vivax* [53]. In Asia, *P. vivax* and *P. falciparum* are the predominant species [54]. In India, isolate *P. vivax* infection is widespread in the southern state of Tamil Nadu, while mixed-species infections are prevalent in the west [44]. In Bangladesh, samples collected from febrile patients underwent PCR research of *Plasmodium* species, showing a prevalence of *P. vivax* infection of 15, 3% in mono-infected patients and of 27,5% in mixed infections [46]. In Cambodia, prevalence of *P. vivax* infection detected by PCR method in samples collected in September 2001 was 15% [47]. Studies performed in Thailand and in Myanmar showed that *P. vivax* is the most prevalent malarial species [48, 49]. Such as in the WHO Eastern Mediterranean Region, in particular in Afghanistan, Islamic Republic of Iran and Turkey [55-58]. Central and South America *P. vivax* is the predominant *plasmodium* species, accounting for 71–81% of all malaria cases [50, 52]. Studies demonstrate that *P. vivax* accounts for 83, 7% of malarial infections in Brazil [59], for 70% of infections in Colombia [60] and for 90% of infections in Ecuador [61].

3. Plasmodium ovale:

The real burden of *P. ovale* malaria is difficult to assess because its diagnosis is difficult. *Plasmodium ovale* may be encountered in sub-Saharan Africa and in Asia [62]. A recent study from Mozambique tested malaria prevalence among febrile patients: only 2 of 111 malaria positive patients presented *P. ovale* mono-infection, while 4 *P. ovale* and *P. falciparum* mixed infections were also detected [63]. In Congo, a cross-sectional, population-based cluster household survey of adults aged 15–59 years demonstrated that *P. ovale* parasitaemia was rare and its prevalence in mono-infection was only 0, 1% [64]. In a recent multicenter study, blood samples were collected from the indigenous population of nine African countries and malaria parasites were searched by PCR method. Of 1.737 samples, 67 were positive for *P. ovale:* 12 single infections, 51 mixed with *P. falciparum* and 4 triple infections with *P. falciparum* and *P. malariae*. When samples from Rwanda, Mozambique, Angola and Sao Tome were excluded, *P. ovale* infection represented 3,9% of all malaria cases [51]. Another study performed in Congo-Brazzaville, Uganda and Equatorial Guinea, concluded that two *P. ovale* species, *P. o. curtisi* and *P. o. wallikeri*, is both widespread in Africa; in Uganda and Equatorial Guinea the prevalence of *P. ovale* spp. in population-based samples was found to be between 1% and 6% [65]. There are many evidence of *P. ovale* infection in Asia. Samples collected during 2007/2008 in Bangladesh from 379 febrile patients who underwent microscopic, DNA extraction and nested PCR analysis: 3 of the 189 positive samples (1, 6%) were positive for *P. ovale* [46]. *Nested* PCR detected *P. ovale* parasites in 1, 3% of blood samples collected from 1.356 inhabitants of eight villages of Rattanakiri Province (Cambodia) [47]. In a study performed in Myanmar, *P. ovale* was detected by PCR technique in 4,9% of malaria positive samples; most of cases were co-infections with *P. falciparum*, *P. vivax* and/or *P. malariae* [66]. Case reports of *P. ovale* infection were recently published from Gujarat, India [67]Malaysia [68]and Sri Lanka [69] *P. ovale* infection is present in Papua, Indonesia [70] and in Thailand [48], while it is very rare in Philippines, where has been reported only in the island of Palawan[71].

4. P. malariae:

P. malariae is spread in sub-Saharan Africa, in Southeast Asia, in Indonesia, in many islands in western Pacific and in areas of the Amazon Basin of South America. Its distribution overlaps with that of *P. falciparum* [72]. In a recent study, blood samples were collected from the indigenous population of nine African countries and malaria parasites were searched by PCR method. *Plasmodium malariae* was found in 147 of the 1.737 positive blood samples, 14 as mono-infections, 129 as mixed infections with *P. falciparum* and 4 as triple infections with *P. ovale* and *P. falciparum* [41]. Excluding samples from Rwanda, Mozambique, Angola and Sao Tome, *P. malariae* infections represented 8.5% of all malaria infections [41] In Nigeria, between November 2001 and October 2002, a total of 350 pregnant women attending the antenatal clinics were randomly recruited and blood samples were collected. Of 350 blood samples, 96 (27,4%) were positive for malaria parasite and 11 (11,5%) were *P. malariae* positive as tested by microscopy [43]. During the rainy season, blood samples were collected from resident people of four villages in Mulanda, Uganda. Of 709 malaria positive samples, 6% were positive for *P. malariae* [42]. In the state of Orissa, India, the prevalence of *P. malariae* detected by PCR method was 44,6% during the peak season of malaria incidence [45]. A recent case report was published demonstrating the presence of *P. malariae* in Bangladesh [73]. In Papua New Guinea, prevalence of *P. malariae* was detected by *nested* PCR, by quantitative PCR and by PCR-ligase detection reaction-fluorescent microsphere assay (PCR-LDR-FMA); the results were 3,3%, 4,7% and 7,7% respectively [74].

5. P. knowlesi:

Plasmodium knowlesi infection is localized only in the South East Asia Region and interest both monkeys, where it was first reported, and humans. Forest areas are the *reservoirs* of *P. knowlesi*, which was first reported in humans in 1965 in a man who had worked in the jungle of Pahang, Peninsular Malaysia. An analysis of stored blood films identified cases of *Plasmodium knowlesi* infection occurring since 1996 in Sarawak region, Malaysian Borneo [75]. In Sabah region, Malaysian Borneo, samples were collected from February to November 2010 from patients with suspected malaria. *Nested* PCR was performed in all 243 samples collected; of 107 samples positive for malaria parasite, 63 were positive for *P. knowlesi*, demonstrating a high incidence of *P. knowlesi* infection in the interior division of Sabah [76]. Another study performed in Thailand screened 1.874 samples collected from febrile patients during a period of one year. Of the 1.751 sample positive for malaria parasites, 10 were positive for *P. knowlesi,* that mostly occurred in males with uncomplicated malaria coming from southern and southwestern regions of Thailand [48] *P. knowlesi* mono and mixed infection were also demonstrated in Myanmar [49] in the Philippines [77] and in Singapore [78] According to a three years prospective study performed in Cambodia, two *P. knowlesi* malaria cases were diagnosed by *nested* PCR[79] A recent study performed in Vietnam showed the presence of *P. knowlesi* in three of ninety-five samples of patients with *P. malaria* mono or mixed infection, demonstrating for the first time the presence of *P. knowlesi* malaria in Vietnam [80].

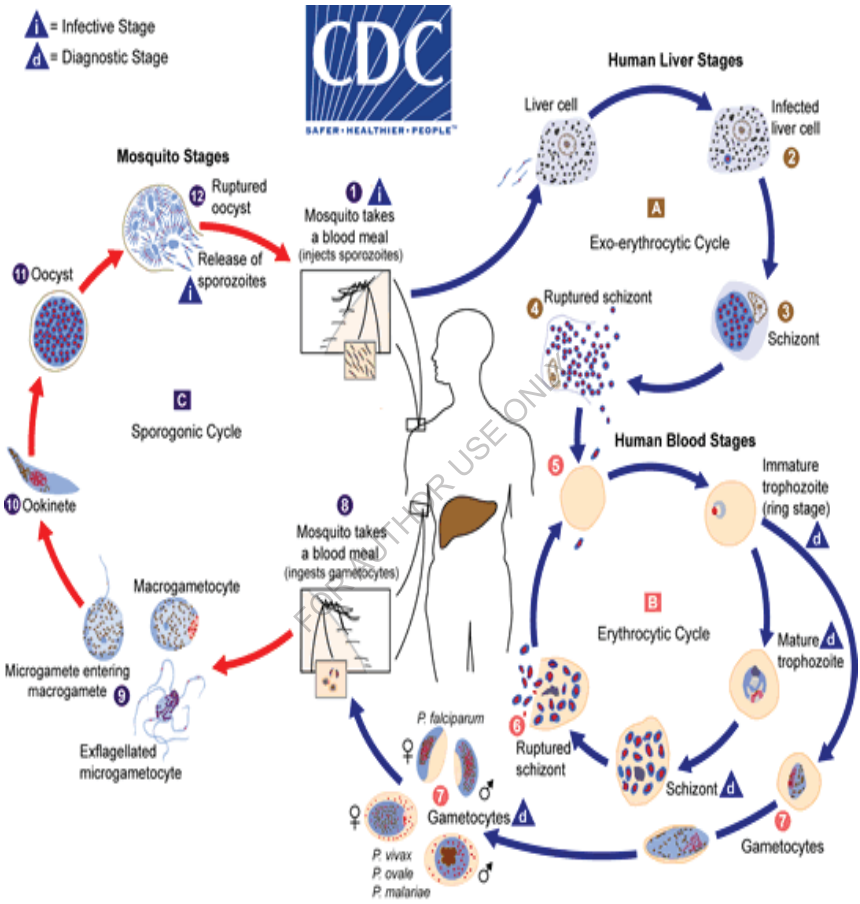
Biology of malaria parasite

Lifecycle:

The natural history of malaria involves cyclical infection of humans and female *Anopheles* mosquitoes. In humans, the parasites grow and multiply first in the liver cells and then in the red cells of the blood. In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites ("merozoites") that continue the cycle by invading other red cells.

The blood stage parasites are those that cause the symptoms of malaria. When certain forms of blood stage parasites (gametocytes, which occur in male and female forms) are ingested during blood feeding by a female *Anopheles* mosquito, they mate in the gut of the mosquito and begin a cycle of growth and multiplication in the mosquito. After 10-18 days, a form of the parasite called a sporozoite migrates to the mosquito's salivary glands. When the *Anopheles* mosquito takes a blood meal on another human, anticoagulant saliva is injected together with the sporozoites, which migrate to the liver, thereby beginning a new cycle.

Thus the infected mosquito carries the disease from one human to another (acting as a "vector"), while infected humans transmit the parasite to the mosquito, in contrast to the human host, the mosquito vector does not suffer from the presence of the parasites.



The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host ❶. Sporozoites infect liver cells ❷and mature into schizonts ❸, which rupture and release merozoites ❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver (if untreated) and

cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells 5. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites 5. Some parasites differentiate into sexual erythrocytic stages (gametocytes) 7. Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal 8. The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes 9. The zygotes in turn become motile and elongated (ookinetes) 10 which invade the midgut wall of the mosquito where they develop into oocysts 11. The oocysts grow, rupture, and release sporozoites 12, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites 1 into a new human host perpetuates the malaria life cycle [81].

Animal models of malaria have provided and will undoubtedly continue to provide important insights into the immunobiology of *Plasmodium* infection [82], but ultimately, despite obvious experimental limitations, it is critical to investigate the immune response to *Plasmodium* infection in humans. In contrast to what is typically available to researchers of other infectious diseases, the malaria research community has access to longitudinal 'models' of both experimental and natural *P. falciparum* infection in humans [83-85]. Under strictly controlled conditions, volunteers are exposed to the bites of laboratory-reared *P. falciparum*-infected mosquitoes. In previously unexposed individuals, parasites become detectable in the blood by microscopy approximately 11 days after infection at which point curative anti-malarial therapy is administered. Since the 1980s over 1500 volunteers have been experimentally infected with *P. falciparum* without serious adverse events, although *P. falciparum*-naïve volunteers often experience transient malaria symptoms such as fever, chills, headache and myalgias. A model of direct intravenous administration of iRBCs in healthy volunteers has also been developed allowing investigations of the early immunological responses to blood-stage infections while strictly controlling the size of the blood-stage inoculum [84]. The highly controlled and predictable nature of human experimental *P. falciparum* infections allows for high-resolution immunological analyses during the skin, liver and early blood-stages of the parasite life cycle.

The study of immune responses to natural infection in individuals of all ages is also feasible in malaria endemic areas due in part to the patterns and intensities of *P. falciparum* transmission. Transmission patterns vary across endemic areas from sporadic to seasonal to year round depending on the temperature, rains and mosquito breeding, and transmission intensities vary from low to high. It is possible to take advantage of these variations to design field-based immunological studies tailored to these transmission patterns and intensities. For example, in areas of intense seasonal malaria, such as in much of West Africa, the intensity of transmission is so great that every individual will predictably be exposed to hundreds of infectious mosquito bites over the course of a transmission season. Cohorts can be enrolled during the six month dry season during which there is no malaria transmission and then followed through the malaria season. The predictable timing and intensity of *P. falciparum* transmission from year to year allow for multi-year cohort studies in which *P. falciparum* infections and clinical malaria episodes can be reliably detected through active parasitological and clinical surveillance and relevant biospecimens can be collected at time points before, during, and after asymptomatic and symptomatic *P. falciparum* infections [85].

Importantly, such designs allow study subjects to serve as their own healthy pre-infection controls and permit two general types of questions to be addressed: 1) What immune parameters or profiles correlate prospectively with protection from malaria?, and 2) How do acute and chronic *P. falciparum* infections modulate the human immune response?.

The immune response to the skin and liver stages of Plasmodium infection:

In humans, the skin and liver stages of *P. falciparum* infection are clinically silent as they fail to induce significant dermal, hepatic or systemic inflammation. Not unexpectedly, the lack of a robust innate immune response to the skin and liver stages is associated with a correspondingly muted induction of antibody, $CD4^+$ and $CD8^+$ T cell responses to the infecting sporozoites in individuals exposed to natural *P. falciparum* infections in endemic areas [86]. Accordingly, there is no convincing evidence for naturally-acquired immunity capable of completely neutralizing the parasite at the skin or liver stages. Indeed, even after decades of repeated *P. falciparum* exposures, adults in malaria-endemic areas are at the same risk of becoming infected with parasites as young children [87]. The cellular and molecular basis of this clinical silence and paucity of sterilizing immunity is only poorly understood. The relatively weak innate and adaptive immune response to the skin and liver stages may reflect the low inoculum of parasites in the mosquitoes' saliva (10 – 100 sporozoites). The inherent immune-regulatory environment of skin [88] and liver [89] *,* may also be exploited by the parasite to evade immune mechanisms. Normal skin has a particularly high proportion of regulatory T cells (Tregs) in the steady state [88] and a metaanalysis of malaria vaccine studies suggested that Plasmodium-specific Tregs are induced during the skin stage of infection, potentially mediating immune tolerance to sporozoites as well as to the subsequent blood-stage of infection [90]. Consistent with these results are experiments in mice that show that skin Tregs and DCs are mobilized within 30 minutes of sporozoite inoculation and that DCs down regulate MHC II and CD86 suggesting a tolerogenic response [31]. The immunoregulatory environment of the skin may be reinforced by chronic immune activation or immune dysregulation due to repeated exposures to sporozoites and liver stage antigens [92]. Plasmodium-specific mechanisms that disable innate immune defenses, such as sporozoite-mediated disruption of Kupffer cells' respiratory burst in the liver [93] may also affect pre-erythrocytic immunity.

In striking contrast to the lack of pre-erythrocytic immunity in natural infection, sterilizing immunity to Plasmodium infection can be readily induced in mice [94]), non-human primates [95] and humans [96] through exposure to radiation-attenuated (RA) sporozoites that are able to infect hepatocytes but cannot replicate in the hepatocyte and do not give rise to blood-stage infections. Sterilizing immunity can also be induced by exposure to genetically attenuated parasites [97] which also invade hepatocytes but do not replicate to produce blood-stage merozoites, and by exposure to sporozoites under chloroquine anti-malarial chemoprophylaxis [98]. Chloroquine kills only blood-stage parasites and thus exposure to sporozoites under chloroquine treatment allows full exposure to sporozoites and liver-stage parasites and only transient exposure to blood-stage parasites. The number of RA sporozoites and route of exposure to RA sporozoites appears critical to induce immunity. Early studies showed sterilizing immunity to RA sporozoites required the bites of over 1,000 infective mosquitoes. A recent trial in humans showed that subcutaneous RA sporozoite immunization did not confer protection [99], however, intravenous RA sporozoite immunization in animal models was protective [99].

In humans, RA sporozoite exposure induces antibodies, CD4+ and CD8+ T cells that react to several sporozoite and liver stage antigens [100-102] , but the relative contribution and antigen specificity of these putative effectors mechanisms remains unclear. Volunteers exposed to sporozoites under chloroquine treatment develop complete protection against homologous

sporozoite challenge (38) that lasts at least 28 months [103]. Exposure elicits both antibody and T cell responses against sporozoite and blood-stage antigens [98]. And protection has been linked to pluripotent effectors memory T cells producing interferon-γ (IFN-γ), tumor necrosis factor (TNF), and interleukin-2 (IL-2) [98] that are most likely directed against liver rather than blood stage antigens [104].

The response to blood-stage infection:

The clinical manifestations of malaria are caused by asexual blood-stage parasites as they replicate in the blood. Although complex and incompletely understood [105], malaria pathogenesis is generally thought to be driven by two distinct processes: sequestration and inflammation. Sequestration involves the binding of iRBCs to receptors on vascular endothelium via specific parasite-host interactions, causing microvascular obstruction, local ischemia and inflammation in the brain and other vital organs [106,107]. P. falciparum also induces a systemic inflammatory response akin to bacterial sepsis [108] Which may exacerbate iRBC sequestration by up regulating vascular adhesion molecules such as ICAM-1 [109]

In areas of intense P. falciparum transmission, resistance to severe life-threatening malaria (discussed below) is generally acquired by the age of five years, whereas children remain susceptible to repeated bouts of febrile malaria through adolescence, eventually achieving near complete clinical immunity to blood-stage parasites by adulthood. Here we focus on two immunological processes that play crucial roles in controlling P. falciparum blood-stage parasites and the disease they cause: inflammation and its regulation, and the antibody response. In previously unexposed individuals, blood-stage P. falciparum parasites invariably induce fever and other signs and symptoms of malaria [110] a process driven by the production of proinflammatory cytokines and chemokines such as IL-1β, IL-6, IL-8, IL-12(p70), IFN-γ and TNF [111-113]. A handful of Plasmodium proteins have also been identified as drivers of inflammation through specific interactions with host receptors. For example, particular PfEMP1s have been recently implicated in parasite sequestration in brain endothelium in severe cerebral malaria in children [114-116] .The receptor for these PfEMP1s was recently identified as the endothelial protein C receptor (EPCR) that mediates cytoprotective effects through activated protein C suggesting that PfEMP1 binding to EPCR may block its protective function and contribute to the pathology of cerebral malaria [117]. Merozoite surface protein 1 (MSP1) that is shed from the merozoite surface as the parasite invades RBCs was shown to bind to S100P a member of the pro-inflammatory S100 protein family and to block S100P activity [118]. These studies were recently extended to show MSP1 binds to all S100 family members (Waisberg unpublished observation). The extent to which heterogeneity in the host inflammatory response and disease severity is the result of polymorphisms in these and other proteins, the expression of specific subsets of PfEMP1s, or variability in the structure, configuration or quantity of Plasmodium PAMPs is an area that requires further study.

Work in animal models demonstrates a role for various innate immune cells in sensing early blood-stage infection, promoting inflammation, inhibiting parasite growth and shaping adaptive immune responses [119] including mast cells [120], neutrophils [121] NK cells, NKT cells and γδ T cells [122]. Human NK cells are a particularly important early source of IFNγ in response to iRBCs in vitro, a central cytokine in the immune response to malaria that promotes the

destruction of iRBCs by activated macrophages. In humans, γδ T cells contribute to early IFNγ production to a lesser degree, while later IFNγ production is dominated by αβ T cell [123].

On the one hand, Plasmodium-induced inflammation likely plays an important role in the early control of parasite replication, for example, IFN-γ and TNF kill blood-stage P. falciparum parasites through the induction of NO and other toxic radicals [124], but on the other hand excessive inflammation has been linked to severe and fatal malaria [125].Which occurs in a minority of individuals who have little or no prior malaria exposure [126]. However, in endemic areas where individuals are repeatedly infected, blood-stage infections commonly cause a mild febrile illness or no symptoms at all [127], consistent with the longstanding hypothesis that tolerance to Plasmodium-induced inflammation can develop [128]. Transcriptome analysis of leukocytes from healthy children before the malaria season and the same children seven days after treatment of their first febrile malaria episode of the ensuing season (when malaria symptoms had resolved) showed that key mediators of P. falciparum-induced inflammation and fever (IL-1β, TNF, IL-8) are down regulated following treatment relative to the healthy pre-malaria baseline. Stimulation of these children's leukocytes in vitro with P. falciparum iRBCs at the same time points showed down regulation of proinflammatory cytokines/chemokines and up regulation of the anti-inflammatory cytokines TGF-β and IL-10 seven days after treatment relative to the healthy baseline.

In addition to genetic polymorphism and antigenic variation, there is mounting evidence that Plasmodium evades humoral immunity through dysregulation of CD4+ T cell and B cell function. It is well established that long-lived protective antibody responses depend on the generation of memory B cells (MBCs) and long-lived plasma cells (LLPCs), a process which relies on CD4+ T cell help [129]. Although there is heterogeneity in the magnitude, quality, and longevity of antibody responses following infection or vaccination, in general, antibody responses are long-lived, even after one or a few exposures [130].

Malaria-associated B cell dysregulation may be driven by direct interactions between P. falciparum products and B cells. For example, the Cysteine-rich interdomain regions 1α of PfEMP1 have been implicated as a T cell–independent polyclonal B cell activator and Ig binding protein [131]. Moreover, chronic exposure to P. falciparum PAMPs could possibly result in tolerance of PRRs expressed on B cells and DCs, which play a critical role in enhancing B cell responses. Indeed, recent clinical trials show that the TLR9 agonist CpG enhances the IgG and MBC response to blood-stage vaccine candidates in malaria-naive adults [132] but not in adults chronically exposed to P. falciparum [133].

The immune response in severe malaria:

Each year there are approximately 225 million cases of malaria in Africa, the vast majority of which are uncomplicated and are resolved with time even without treatment with antimalarial drugs. However, in a small portion of cases, approximately 1% almost exclusively in young children or in older individuals with little or no prior exposure, the infection becomes severe and life threatening resulting in nearly a million deaths each year [134]. Malaria during pregnancy can also be severe resulting in substantial maternal, fetal and infant morbidity. Because of space limitation we focus our discussion to severe malaria in young children and refer the reader to an

excellent review on immunity to malaria in pregnancy [135]. Most severe malaria deaths in children are due to three overlapping clinical syndromes, malaria with impaired consciousness (cerebral malaria), malaria with respiratory distress due to severe metabolic acidosis and severe anemia [136].

In children the pathology of severe disease has been consistently linked to excessive inflammatory responses including the production of the pro-inflammatory cytokines TNF-α, INF-γ, IL-1β and IL-6 and a linked reduced production of the antiinflammatory cytokine IL-10. At present we do not have a clear picture of the sequence of events responsible for the progression from uncomplicated malaria to severe disease or of the events that trigger severe disease. Currently, it is not possible to distinguish children who are at high risk to progress from uncomplicated to severe malaria and we have no specific therapies that are effective for children who present with severe disease. Clearly a better understanding of the immune mechanisms at play in severe disease would contribute to efforts to development of such diagnostics and therapies.

Because severe disease is difficult to study in children, most investigations of the cellular and molecular basis of severe malaria have been restricted to mouse models, in particular, a mouse model of cerebral malaria. At present, the value of the mouse cerebral malaria model to understanding cerebral disease in children is controversial [137].

Mosquito immunity and malaria

The lack of adaptive immunity in insects, the relatively simple organization of their immune system and the ability to disrupt gene function in adult stages makes them powerful models to understand the basic molecular mechanisms that mediate innate immune responses [138]. This is particularly true of anopheline mosquitoes, the natural vector of malaria, as models of anti-plasmodial responses. Understanding the interactions of the mosquito immune system that affect Plasmodium parasites is critical to elucidate the factors that sustain transmission in the field, but can also provide new insights into molecular mechanisms that may also be operating in the human host

Pathology of malaria parasite

All the clinical symptoms of malaria are the consequence of infection of human erythrocyte by merozoites. Most of the fatal cases, which predominantly occur in *P. falciparum* infections, are due to severe anaemia or cerebral malaria, but different clinical manifestations also exist and vary in severity and outcome, depending on the parasite species, the organ involved and the access to care.

P. falciparum differs from other human malarial species in that infected red blood cells (IRBC) do not remain in the circulation for the entire life cycle. After 24–32 hours, when young parasites mature from the ring to the trophozoite stage, IRBC adhere to endothelial cells in the microcirculation of various organs. This phenomenon, termed "sequestration", is believed to occur mainly to avoid splenic removal of IRBC. Sequestration causes microcirculatory obstruction, impaired tissue perfusion and inflammatory cells activation and it is linked to the severity of the disease.

At schizonts rupture, from 4 up to 36 daughter merozoites, depending on the Plasmodium species, are released into the circulation and invade fresh RBC to perpetuate the asexual life cycle. At the same time, a large amount of toxins and parasite products are also released and cause the activation of the innate immunity, the release of inflammatory mediators and the symptoms associated with the malaria attack, such as fever.

A combination of mechanical circulatory stress due to sequestration and excess inflammatory response contribute to the most severe manifestations of malaria including, but not limited to, cerebral malaria or anaemia. The present review will summarise some of the pathogenetic aspects of severe malaria and then it will report on selected and less frequent manifestation, such as renal failure or acute respiratory distress syndrome (ARDS).

Pathogenetic Characteristics of Severe Malaria:

Cytoadherence:

Cytoadherence, the ability of parasites to adhere to the vascular endothelium, was recognized as early as 1892 by Marchiafava and Bignami [139]. Mature forms of parasites (asexual stage and gametocytes) can adhere to the vascular endothelium of several organs (lung, heart, brain, lung, liver, and kidney), the subcutaneous adipose tissues and the placenta. This feature of the disease *in vivo* has been related exclusively to *P. falciparum* [140,141]. However, sequestration *in vitro* to some endothelial cell lines and placental cryosections has also been seen in reticulocytes infected with *P. vivax* [142].

Parasite sequestration is thought to be the pathological base of the severe manifestation of malaria, including cerebral malaria [143].It causes blood flow impairment leading to local

hypoxia. It enhances parasite replication and the sticking of IRBC to non-infected red blood cells (rosetting, see below). Moreover, when parasite sequester, the effects of parasite toxins are more localized and also the stimulation of the host immune response, which may cause a focused production of inflammatory mediators and tissue damage. As a consequence, both RBC and IRBC become more rigid and less deformable [144].

Sequestration is mostly mediated by mature parasite forms, approximately 20 hours after RBC invasion. The parasites produce new proteins that are exported to the IRBC surface and increase the adhesiveness of IRBC to the endothelium. During their 48-hour life cycle, the parasites can remain sequestered for 24 hours in the deep microvasculature. In this manner, they evade clearance by the spleen, and make the diagnosis more difficult since they are not seen in the peripheral blood.

Sequestration of *P. falciparum* has been attributed to different class of molecules of parasite origin and ligands present on the human endothelium. Among those, the *P. falciparum* histidine-rich protein (PfHRP) and the erythrocyte membrane protein 1 (PfEMP1), have received significant attention. PfHRP is related to the establishment of knobs, symmetric membrane arrangements which appear on the surface of infected RBC, while PfEMP1, a multimeric protein encoded by the *var* (variant) gene [140,141] protrudes from the knobs and plays a major role in sequestration and thus virulence. To adhere to the endothelium, the parasites first adhere, roll and then become firmly attached to the endothelium adhesion molecules. Among these molecules, ICAM-1, a major sequestration receptor and involved in cerebral sequestration serves as a rolling receptor. On the other hand, CD36 gives stationary and stable adherence under flow [145-147].

Sequestration is also seen during gestational malaria, when parasites adhere to the placenta. PfEMP1 is again the main adhesion receptor which adheres to the trophoblastic villous endothelium mainly through chondroitin-4-sulfate (CSA) and other sugars such as glycosaminoglycans and possibly hyaluronic acid (HA). As discussed later, malaria in pregnancy can be severe for mothers and induce fetal death especially during the first pregnancy, when women usually lack sufficient immunity against CSA-binding parasites [148-151].

Rosetting:

Rosetting is one of the forms of cytoadherence of late stages IRBC to non-parasitized red blood cells and/or platelets [152]. The IRBC ligand involved in rosette formation is PfEMP1, and three are the receptors associated with rosetting: complement receptor 1 (CR1), heparan sulfate (HS), and the ABO blood group [152,153]. PfEMP1 has been shown to bind to CR1, specifically at the C3b-binding site. The lectin-like DBL-domain of PfEMP1 can make strong adhesion with carbohydrate structures particularly A blood group antigen, favoring rosettes formation [154] For this reason, non-O blood groups are considered significant risk factors for life-threatening malaria, through the mechanism of enhanced rosette formation [155,156].

P. falciparum, P. vivax, and *P. ovale* are all able to form rosettes [157,158] but only those caused by *P. falciparum* have been associated with severe malaria, and especially in African children they may enhance microcirculatory obstruction [159]. Rosetting has been related with parasite multiplication rate in a model of *Saimiri sciureus* [160]. More recently, it has been shown that 4-

HNE, a biomembrane lipid peroxidation product driven by haem iron of the malarial pigment can be transferred from IRBC to normal RBC in rosettes favouring their removal by macrophages [161]. This could partly explain the rapid loss of normal non parasitized RBC in severe malaria anaemia.

Specific Complications of Severe Malaria Infection:

Anaemia:

Anaemia is one of the most common causes of morbidity and mortality in malaria infection particularly in pregnant women and in children [162]. Pathogenesis of malarial anaemia has been intensively studied, even if it is not completely understood; hereby we summarise some of the pathogenetic aspects. Malarial anaemia could be acute or chronic; in holo-endemic areas chronic malarial anaemia is more common. Acute malarial anaemia could occur after massive erythrocytes lysis due to elevate parasitaemia or to drug-induced or immune hemolysis [163].

The potential mechanisms contributing to malarial anaemia can be divided into two categories: increased destruction of parasitized and un-parasitized erythrocytes (immune-mediated lysis, phagocytosis splenic sequestration) and decrease of RBC production (dyserythropoiesis and bone marrow suppression, inadequate reticulocyte production, effects of inflammatory cytokines, effects of parasite factors). Co-infection with bacteremia, HIV-1 and hookworm, malnutrition and repeated malarial infections in holoendemic countries may also contribute to decrease hemoglobin levels [162,164-166].

Dyserythropoiesis plays an important role in the pathogenesis of anaemia; examination of bone marrow from children with severe anaemia showed hypercellularity, mild to normal erythroid hyperplasia and abnormal features of late erythroid progenitors. Hemozoin and its phagocytosis by bone marrow macrophages has been proposed to cause dyserythropoiesis either through direct accumulation in the bone marrow and generation of reactive toxic species or activation of the innate immune response [165].

The immune response is central in the pathogenesis of malarial anaemia; parasitized red cells, hemozoin and malarial antigens activate monocyte and lymphocyte response. Pro-inflammatory and antiinflammatory mediators, including TNF-α, IFN-γ, IL23 and IL-1, chemokines and growth factor are produced and contribute to anaemia. On the contrary, IL-12 and IL-10 seems to be protective cytokines since low levels are found in severe malarial anaemia [163,166]. Macrophage migration inhibiting factor (MIF) is associated with severe anaemia and bone marrow suppression.47 Nitric oxide is an inhibitor of erythropoiesis [163,167].

Erythropoietin (EPO) levels are increased during malaria anaemia, but erythroid progenitors response is not adequate, particularly during chronic malaria infection, resulting in low reticulocytosis [167].

Pro-inflammatory cytokines play an important role also in iron delocalisation pathway of malarial anaemia. TNFα induces re-localisation of ferroportin, an important protein abundant in the reticuloendothelial system that mediates macrophage iron release and intestinal iron

absorption. Relocalisation of ferroportin induces decrease of iron absorption and release from macrophage cells [168], hepcidin, a protein released during chronic disease, also induces reduction of ferroportin and its levels are increased during severe malaria anaemia [169].

Thrombocytopenia:

Thrombocytopenia is very common in malaria, usually during the early stage of P. falciparum and P. vivax infections. Incidence is high both in children and in adults [170-172]. Thrombocytopenia in pregnant women is not well documented, but a recent study performed in Thailand showed that platelet counts were lower in pregnant than in nonpregnant women [173].

The pathogenesis of malaria thrombocytopenia is complex and may be related to coagulation disturbances, splenomegaly and platelet destruction by macrophages, bone marrow alterations, antibody-mediated platelet destruction, oxidative stress and platelets aggregation [174].

A study performed in Indonesia showed that patients with P. falciparum and P. vivax malaria had lower platelet count, higher von Willebrand factor (VWF) concentration, lower ADAMTS13 activity and ADAMTS13 antigen concentrations [175]. Higher VWF seems to correlate with platelet binding, leading to thrombocytopenia. In contrast, another study demonstrated that sGP1b, the external domain of platelet receptor for VWF, increased early in the blood of malaria patients thus preventing excessive platelet adhesion [176].

Acute respiratory distress syndrome:

Deep breathing, respiratory distress and pulmonary oedema are some of the clinical feature defining severe malaria according with WHO classification.64 In adults and pregnant women, rather than children, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are the most common clinical presentation burdened by an elevated mortality rate as shown in a recent study performed in India where severe P. falciparum malaria mortality rate was 35,4% and mortality was principally due to shock, acute renal failure, seizure and ARDS [177].

Different pathological presentations of ARDS were observed among the different species of human malaria: the greatest severity and frequency of cases are due to P. falciparum and could be partially attributed to the sequestration and rosetting of infected RBC in the pulmonary microcirculation [178]; heavy parasitaemia and WBC agglutinates were associated to ARDS in P. vivax malaria [179].

Liver involvement:

According to the World Health Organization liver dysfunction is an uncommon occurrence in malaria, while jaundice is not unusual. The incidence of jaundice and liver dysfunction in P. falciparum malaria varied from 5.3% to 62% and from 2.5% to 21%, respectively, in different reports [180-182], while malarial hepatitis is rare in P. vivax infection [183]. Case-fatality rate in malaria-related hepatic failure is elevated, up to 40% [182-184], when high parasite density is associated with jaundice and liver dysfunction [185].

Liver is involved in malaria at two stages: during the preerythrocytic cycle and the erythrocytic phase. The first step is linked to the binding of the merozoite circumsporozoite protein CSP-A and TRAP protein to the hepatocytes via the heparan sulphate glycosylaminoglycans GAG [186]. Intravascular hemolysis of parasitized and non parasitized RBC causes an increase of unconjugated bilirubinemia with mild to moderate jaundice [187]; conjugated hyperbilirubinemia indicates hepatocyte dysfunction.

Kidney involvement:

Kidneys in malaria are involved in two different manners: acute and chronic diseases. Acute renal failure (ARF) is one of the most challenging diseases in tropical countries and malaria plays an important epidemiological role [188-191].

Quartan malaria nephropathy (QMN) is frequently seen in African children and it is clinically associated with oedema and hypertension; urine analysis shows often proteinuria and microhematuria [192,193].

Black water fever (BWF), a rare but severe complication of severe malaria, is characterized by fever, intravascular haemolysis with haemoglobinuria, dark urines and often acute renal failure [194-196]. Haemoglobin release during massive haemolysis causes renal impairment. Drugs as quinine, halophantrine and mefloquine, and G6PD deficiency, have been suggested to be the trigger of BWF.

Placenta involvement:

Malaria during pregnancy is associated to high morbidity and mortality both for the mother and the child [197-200]. Placental malaria is quite common during P. falciparum infection; less common in P. vivax malaria; P. falciparum and P. vivax placental mixed-infection can occur [201].

In high endemic areas, while adults are less susceptible, pregnant women are commonly susceptible to malaria infection because pregnancy causes a transient depression of cell mediated iAlterations of materno-fetal blood exchange are the basis of placental malaria.mmunity. During infection, parasitized red cells both from P. falciparum and P. vivax are sequestered within the placenta and they accumulate in intervillous spaces; trophozoite and schizont forms also accumulate in the placenta[150]. The presence of IRBC activates mononuclear cells which release chemokines to recruit additional phagocytic cells in the intervillous spaces. Elevated TNF α and IL-10 levels were also described and were associated with poor pregnancy outcomes [150].

IRBC, leukocyte infiltration, fibrin and hemozoin depositions contribute to increase the thickness of the trophoblast basement membrane and to alter the intervillous and perivillous spaces, causing reduction of oxygen and nutrient transport to the fetus [150,202].

The pathogenicity of severe malaria infection is complex and it is regulated by both parasite and host factors. Cytoadherence of IRBC to the vascular endothelium and rosetting are unique features of malaria parasites which are likely to contribute substantially to the vascular damage

and the consequent excessive inflammatory/immune response of the host. This can occur in many different organs, a feature that can partly explain the complexity of the clinical manifestations occurring in severe malaria.

Malaria diagnostic methods

At the present time there are a limited number of methods for the diagnosis of malaria. Conventional methods include clinical diagnosis by history and physical examination, empirical/syndromic diagnosis (mainly the presence of fever in endemic areas), and use of light microscopy to examine stained peripheral blood smears. Histopathology plays a limited role, but although it is useful in some situations, it is not useful in malaria control programs. Nucleic acid amplification tests play almost no role in malaria diagnosis, as these assays are limited to a few large public health laboratories and are not available commercially. As with other common infectious diseases, a number of rapid diagnostic tests have been developed and marketed. Referred to as MRDTs, they potentially could have the most impact on malaria diagnosis and treatment programs of all the available diagnostic techniques.

Empiric/Syndromic Diagnosis:

One widely used method to diagnose malaria is empiric/syndromic diagnosis, in which the diagnosis is made on the basis of clinical history, signs, and/or symptoms. In many endemic areas without adequate diagnostic capacity, patients with a febrile illness are likely to receive the diagnosis of malaria. There are a number of pitfalls associated with this approach. First, there is significant clinical overlap among febrile illnesses; fever alone is too nonspecific to make any particular diagnosis. Second, coinfections can and do occur, and treatment for one obviously does not treat the other. Third, malaria parasitemia can occur that is not the cause of the febrile illness. Last, relying on clinical diagnosis alone results in treatment of patients with antimalarial drugs for illnesses other than malaria [203] The WHO recommends against this practice when and where malaria diagnostic tests are available [204].

Microscopic Slide Examination:

In most endemic areas, microscopic slide examination of peripheral blood remains the most widely used test as well as the gold standard for detecting malaria parasitemia. The 2011, WHO report [204], indicates that in 2010 there were a total of 165 million microscopic slide examinations worldwide. Estimates of diagnostic sensitivity of microscopic slide evaluation vary according to the type of infecting species, geographic area, and other factors, but in general diagnostic sensitivity is thought to be no higher than 75%. This figure is based on the rate of detection of parasitemia in patients with clinical malaria. For patients with nonfalciparum malaria, low-level parasitemia, or partial immunity, or those who have been partially treated for malaria, the diagnostic sensitivity is likely to be even lower than 75%. Even so, microscopy offers significant advantages over other methods, and, where it can be done correctly and with good quality assurance, it remains the gold standard against which other methods are compared.

Microscopy is based on examination of both thick and thin films made from the same sample of peripheral blood. Thin films are prepared in the same way as for any peripheral blood smear. A number of different stains can be used, but it is important to remember that not all stains allow detection of some of the characteristic features of malaria (eg, Schüffner dots). It is also

important to remember that stains can vary in quality and consistency, and that adequate quality control and experience both are needed to provide optimal stains. Thick films are made by placing a few drops of blood on a glass slide, allowing the blood to dry, and then lysing the blood (usually with water) before staining. Although thick films are more sensitive for detecting the presence of malaria parasites, they are not very useful in speciating the parasites, which should be done using the thin films. Even though the technology of microscopy is simple and straightforward, making and interpreting malaria smears requires adequate training and experience.

The diagnostic advantages of microscopy are that it (1) permits definitive identification of infecting species as well as mixed infections; (2) can be used to determine the magnitude of parasitemia; (3) can be used for serial examinations to monitor the efficacy of therapy; (4) requires little laboratory infrastructure; and (5) is comparatively inexpensive. Microscopic slide examination does have diagnostic disadvantages, including (1) it does not detect very low parasitemias; (2) errors in interpretation are most common with either very low or very high parasitemias (for which accurate diagnosis is very important); (3) mixed infections are often missed; and (4) it is not as useful in areas without endemic malaria because of the inability of persons reading smears to remain sufficiently competent to make accurate and reproducible diagnoses.

Microscopic slide examination also has nondiagnostic advantages and disadvantages. Among the advantages are that the microscope and trained personnel can be used to diagnose other infectious diseases, slides can be retained to create a permanent record for quality control, excess microscope slides can be reused or recycled, and the method requires only minimal laboratory infrastructure. Among the nondiagnostic disadvantages are that the method is labor intensive; it is relatively slow (particularly for thick films); the variable stains and methods result in variable smear quality; the method requires adequate training; and for an area that previously did not use the method, acquiring the necessary equipment and training personnel can be expensive. The difficulty in learning to interpret malaria smears is generally overstated as a limitation, as even individuals without a laboratory background can be trained to read smears in a reasonable amount of time and with good success.

Rapid methods:

Characteristic of an Optimal Rapid Diagnostic Test:

In a recent review of rapid malaria tests, Murray et al [205], described the characteristics of an optimal rapid diagnostic test. Briefly, an optimal diagnostic test would use simple technology; would be readily learned by users; would have results that are easy to interpret (both by users and by providers who ordered the test) and reproducible; would not require electricity to run the assay; would not require refrigerated storage; and, obviously, would be rapid. These characteristics apply to any rapid diagnostic test: the clinical setting where rapid testing is needed, whether for the diagnosis of malaria, human immunodeficiency virus, or tuberculosis, is one where interventions need to be done immediately and without any significant delay.

Additional Characteristics of MRDTs:

In addition to characteristics common to any rapid diagnostic test, MRDTs have additional performance characteristics that are of importance. Most important is the ability to distinguish among species, both because of the prognostic importance of distinguishing between falciparum and other forms of malaria and because of the therapeutic importance of identifying cases of *Plasmodium ovale* or *Plasmodium vivax* malaria, for which treatment with primaquine is needed. The ability to detect mixed infections is of importance for the same reasons. Defining the sensitivity and specificity for detecting each species is important for malaria control programs to be able to select the assay best suited for specific geographic areas.

MRDTs: Current State:

A large number of rapid methods have been developed and marketed, many of which are in clinical use. The most recent WHO report notes that global use of MRDTs has expanded rapidly, particularly in sub-Saharan Africa, the Southeast Asia region, and the Eastern Mediterranean region [204].

Early MRDTs were problematic: (1) there were rapid changes in the assays by manufacturers, often without subsequent controlled clinical trials to evaluate the new versions; (2) inconsistent manufacturing standards existed in many parts of the world; (3) use of MRDTs in the field often was accompanied by inadequate quality control; and (4) assays showed variable product stability once they were shipped from manufacturing facilities [203,205]Even today, in many countries regulatory oversight of manufacturing continues to be inadequate or lacking altogether, and there remain serious concerns about the quality of many of these products [206] Nonetheless, a wide variety of test methods are available commercially: WHO estimates that there are at least 60 brands offering more than 200 commercial MRDTs worldwide [207].

MRDTs: Biochemical Basis:

The currently available MRDTs generally detect malaria-specific protein antigens or enzymes. The most important of these are the *P falciparum* histidine-rich protein 2, which is specific to *P falciparum*, and *Plasmodium* spp lactose dehydrogenase, which may be species-specific or pan-specific (ie, may detect all species). Some MRDTs detect the presence of aldolase, which is not species specific but rather is found in all malaria species.

Most MRDTs currently in use are immunochromatographic strip assays, a technology that allows for mass production of assays. Mass production, in turn, should allow for lower manufacturing and distributing costs, a simplified supply chain, easier training of users, standardization of result interpretation, and easier use in countries or regions where multiple languages are spoken (thereby requiring less complex assays to minimize training and education costs).

The technology is generally similar for most of these assays, although there are some variations. In general, the liquid specimen is applied to one end of the strip, where it mixes with lysing

agents, buffer, and labeled antibody. The labeled antibody then binds to parasite antigens. The fluid mixture then migrates across the nitrocellulose membrane to a point where antibodies fixed on the strip surface can bind to a different set of epitopes on parasite antigens. If the fluid mixture is captured by these fixed antibodies, indicator antigens bound to the labeled indicator antibodies will create a line on the strip, giving a positive test result. It should be emphasized, however, that there are many variations in the exact way immunochromatographic strip assays are designed and used. There are at least 7 broad categories of immunochromatographic strip MRDTs, each containing varying combinations of antibodies to detect different antigens [203,205].

Performance Evaluations of MRDTs:

Clinical evaluations of MRDTs are difficult to conduct and to standardize, whether using microscopy as a gold standard or simply comparing one assay with another. There are a number of reasons for this. First, the epidemiology of malaria in study populations varies substantially: geographic regions even short distances apart may have different types of malaria, varying prevalence and incidence rates, variable access to diagnosis and treatment facilities, and highly variable patient populations because of migration, political instability, or other factors. Second, both malaria-naïve and semi-immune patients live in the same areas, where tests designed to detect the presence of malaria antigens may be positive in patients who have low-level malaria parasitemia but who do not have clinical disease. Third, whether patients have had prior treatment is not always apparent because of the lack of good medical records in many areas. Fourth, antigenemia can persist following adequate treatment, leading to false-positive test results. Because malaria enzymes are cleared faster than antigens, assays that detect enzymes may yield negative test results when compared with assays that detect antigens. Last, environmental factors can affect assays differently, leading to more rapid degradation of one assay compared with another. These factors, combined with the high number of assays currently available commercially, make designing and conducting controlled clinical trials challenging. As a result, other approaches are needed to determine the performance characteristics of MRDTs and to compare different assays against one another.

WHO Evaluations of MRDTs:

Because of the aforementioned difficulties in designing and conducting field trials of MRDTs, the WHO has undertaken extensive evaluations to identify tests that show the best performance characteristics [207].This has been a collaborative effort between WHO, the Foundation for Innovative New Diagnostics, the Centers for Disease Control and Prevention, and the Special Programme for Research and Training in Tropical Diseases. The evaluations were based on a complex study design consisting of 2 parts. The first part of the evaluation was to determine which commercial manufacturers could meet specific quality manufacturing requirements. For products that met this criterion, the second part of the evaluation consisted of testing the MRDTs using stored donor blood seeded with parasites [207]. In the first round, a total of 41 assays from 21 manufacturers were tested using cultured *P falciparum* isolates. In the second round, a total of 29 assays from 13 manufacturers were evaluated. In round 3, a total of 50 assays from 23 manufacturers were evaluated. Of the 120 assays tested, 118 were selected to be evaluated for detection of wild *P falciparum* and *P vivax*, with control testing of blood not seeded with

parasites. Heat stability was also evaluated. Results of this third round of the evaluation were published in the document *Malaria Rapid Diagnostic Test Performance: Results of WHO Product Testing of Malaria RDTs: Round 3 (2010–2011)* [207]. The results of this evaluation showed that, as a group, MRDTs exhibit wide variability in performance at low parasite concentrations, better performance at high parasite concentrations, wide variability among products, and even some variability among lots [207]. It should be emphasized that these evaluations were not field trials, but rather in vitro evaluations of commercial products using seeded specimens. Even so, the results show that only a small number of assays show acceptable performance characteristics and should be considered for clinical trials or procurement programs.

Field Use of MRDTs:

Rapid diagnostic tests have the potential to improve the detection of malaria parasitemia where it is needed most—in the field—but there are practical issues that need to be addressed for MRDTs to be used effectively [203,205].First, there needs to be a supply chain that is appropriate and adequate for the product being used. In some cases this requires temperature control throughout the shipping and storage process. Second, the persons using the tests need adequate training and supervision, and must have readily available written instructions. Third, adequate quality control and quality assurance programs must be in place. Fourth, the tests must be used in context with other components of a malaria control program, and, in particular, there must be the necessary drugs to treat patients based on the results of the test. Fifth, MRDTs must be selected based on the specific needs of a region, in particular the degree of endemicity and type(s) of malaria species present [208]Last, MRDTs must provide test results that are consistent and reliable for local users to gain confidence in them to the point where they will act on test results consistently [209].

MRDTs: Recommended Use:

The current WHO recommendation is that, if there is no other diagnostic support, it is appropriate to use MRDTs. If, however, microscopy is available, both should be used to detect malaria parasitemia. In addition to using 2 tests to improve the case detection rate, the use of microscopy serves other purposes. First, microscopy can be used as a quality control mechanism for MRDTs, to determine both whether the MRDTs are detecting malaria and whether they are detecting the correct species. Second, microscopy is useful when rapid test results are negative, which can occur when a species-specific test does not detect other species. Third, because MRDTs cannot be used to determine the magnitude of parasitemia, microscopy is still needed. Last, microscopy can help with resolution of confusing cases, such as with mixed infections.

Histopathologic diagnosis of malaria:

Despite the primary advantage of histopathology, being able to characterize the disease process, it is not a first-line diagnostic method in malaria control programs. This is for the obvious reasons: the method is insensitive for detecting parasites, identifying species generally is not possible, and the method is too slow and too expensive. Nonetheless, it remains an important diagnostic method for determining the cause of cases of fever of unknown origin in patients from

areas where there are multiple causes of febrile illnesses, it is an important part of the autopsy and determining cause of death, it can play an important role in the quality control of research on malaria diagnosis and treatment, and through all these mechanisms it can contribute to public health epidemiology.

The classic description of falciparum malaria in tissues is that of a small vessel disease due to involvement of capillaries and small vessels [210]. These vessels may be filled with parasitized red blood cells, because of the expression of adhesion molecules on the surfaces of infected red blood cells that adhere to endothelial cells within small vessels. Microscopic examination of tissues from acute cases may show focal necrosis and acute inflammation, and the brain may show ring hemorrhages, but the histopathologic findings may be subtle. More chronic infections may show malarial pigment within cells of the reticuloendothelial system.

Many classic descriptions of the pathology of malaria remain valid, but they may not correlate with current knowledge of pathophysiology, immunity (especially partial immunity), treatment using contemporary drugs, and the synergy of malaria with human immunodeficiency virus infection. Clearly, research in the histopathology of malaria is still needed, particularly in the study of the effects of the disease in women and children. Research in the histopathology of malaria will continue to play an important role in evaluating treatment programs as a quality control mechanism, in helping clarify cause of death in areas where other causes of febrile illnesses are common, and in studying the pathophysiology of the disease.

Nucleic acid amplification tests:

At this time, there are no commercial nucleic acid amplification tests for the diagnosis of malaria. Because developing one of these tests requires expertise that generally is not available in most clinical or hospital laboratories, use of the few assays that do exist is restricted to larger public health laboratories. Not surprisingly, because of the cost of these tests and the long turnaround time to get test results, they are not used in malaria control programs except on a research or epidemiologic basis.

It is likely that some type of automated or point-of-care nucleic acid amplification tests will be developed in the near future, but whether such an assay will have a significant impact on malaria diagnosis is unknown. Issues of cost, requirements for laboratory infrastructure, and other factors will need to be considered carefully in the design, development, and use of such assays.

Cost-effectiveness of malaria diagnostic tests:

As with any diagnostic laboratory test, testing for malaria should always be done using the most cost-effective method for the setting in which it is to be used. In many cases the most cost-effective test may not be the least expensive test. In general, a cost-effective test is one that requires only minimal laboratory infrastructure, requires little training to perform, is easy to use, has easily interpretable results, does not require refrigerated transportation or storage, has a long shelf life, and has sufficient diagnostic sensitivity and specificity to meet the needs of the providers who are ordering the test. For many health care settings, microscopy may be the most cost-effective diagnostic test, particularly in areas where microscopy already is in use and where

introduction of MRDTs is not feasible. In areas where microscopy is not in use, it may be more cost-effective to introduce use of MRDTs without microscopy.

The most important consideration in determining cost-effectiveness of any component of a malaria control program is whether the component reduces morbidity and mortality from the disease. In terms of diagnostic tests, this is determined not only by the performance characteristics of the test, but also by how it is used, whether it is used in such a manner as to yield consistently accurate test results, and whether the results are used to guide appropriate therapy [209,2011].

In one economic model of cost-effectiveness, it was found that, in areas of low to moderate malaria transmission, both MRDTs and microscopy were more cost-effective than presumptive therapy, and MRDTs were more cost-effective than microscopy [209]. As the prevalence of malaria increased, however, presumptive therapy became the more cost-effective approach. In all scenarios, however, it was emphasized that one of the critical factors was a consistent therapeutic approach based on test results. Thus, as with other rapid diagnostic tests, the cost-effectiveness of the test may depend less on the test per se and more on how it is used and what actions are taken based on the results of the test [209]. This will be of particular concern as malaria control efforts become more successful, potentially resulting in more persons with low-level parasitemia. In these patients, MRDTs may be less useful [212].

Treatment of Malaria

Evaluation and diagnosis:

Because malaria cases are seen relatively rarely in North America, misdiagnosis by clinicians and laboratorians has been a commonly documented problem in published reports. However, malaria may be a common illness in areas where it is transmitted and therefore the diagnosis of malaria should routinely be considered for any febrile person who has traveled to an area with known malaria transmission in the past several months preceding symptom onset.

Symptoms of malaria are generally non-specific and most commonly consist of fever, malaise, weakness, gastrointestinal complaints (nausea, vomiting, and diarrhea), neurologic complaints (dizziness, confusion, disorientation, and coma), headache, back pain, myalgia, chills, and/or cough. The diagnosis of malaria should also be considered in any person with fever of unknown origin regardless of travel history.

Patients suspected of having malaria infection should be urgently evaluated. Treatment for malaria should not be initiated until the diagnosis has been confirmed by laboratory investigations. "Presumptive treatment" without the benefit of laboratory confirmation should be reserved for extreme circumstances (strong clinical suspicion, severe disease, impossibility of obtaining prompt laboratory confirmation, usually by microscopy).

Laboratory diagnosis of malaria can be made through microscopic examination of thick and thin blood smears. Thick blood smears are more sensitive in detecting malaria parasites because the blood is more concentrated allowing for a greater volume of blood to be examined; however, thick smears are more difficult to read. Thin smears aid in parasite species identification and quantification. Blood films need to be read immediately; off-hours, qualified personnel who can perform this function should be on-call. A negative blood smear makes the diagnosis of malaria unlikely. However, because non-immune individuals may be symptomatic at very low parasite densities that initially may be undetectable by blood smear, blood smears should be repeated every 12–24 hours for a total of 3 sets. If all 3 are negative, the diagnosis of malaria has been essentially ruled out.

After malaria parasites are detected on a blood smear, the parasite density should then be estimated. The parasite density can be estimated by looking at a monolayer of red blood cells (RBCs) on the thin smear using the oil immersion objective at 100x. The slide should be examined where the RBCs are more or less touching (approximately 400 RBCs per field). The parasite density can then be estimated from the percentage of infected RBCs, after counting 500 to 2000 RBCs.

In addition to microscopy, other laboratory diagnostic tests are available. Several antigen detection tests (rapid diagnostic tests or RDTs) using a “dipstick” or cassette format exist, but only one is approved for general diagnostic use in the United States. RDTs can more rapidly

determine that the patient is infected with malaria, but they cannot confirm the species or the parasitemia. Laboratories that do not provide in-house on-the-spot microscopy services should maintain a stock of malaria RDTs so that they will be able to perform malaria diagnostic testing when urgently needed.

Parasite nucleic acid detection using polymerase chain reaction (PCR) is more sensitive and specific than microscopy but can be performed only in reference laboratories and so results are not often available quickly enough for routine diagnosis. However, PCR is a very useful tool for confirmation of species and detecting of drug resistance mutations. CDC offers malaria drug resistance testing for all malaria diagnosed in the United States free of charge. Serologic tests, also performed in reference laboratories, can be used to assess past malaria experience but not current infection by malaria parasites. Your state health department or the CDC can be contacted for more information on utilizing one of these tests.

Treatment: General Approach:

It is preferable that treatment for malaria should not be initiated until the diagnosis has been established by laboratory investigations. "Presumptive treatment" without the benefit of laboratory confirmation should be reserved for extreme circumstances (strong clinical suspicion, severe disease, impossibility of obtaining prompt laboratory diagnosis).

Once the diagnosis of malaria has been made, appropriate antimalarial treatment must be initiated immediately. Treatment should be guided by three main factors:

• The infecting Plasmodium species •

The clinical status of the patient

• The drug susceptibility of the infecting parasites as determined by the geographic area where the infection was acquired and the previous use of antimalarial medicines.

The infecting Plasmodium species:

Determination of the infecting Plasmodium species for treatment purposes is important for three main reasons. Firstly, P. falciparum and P. knowlesi infections can cause rapidly progressive severe illness or death while the other species, P. vivax, P. ovale, or P. malariae, are less likely to cause severe manifestations. Secondly, P. vivax and P. ovale infections also require treatment for the hypnozoite forms that remain dormant in the liver and can cause a relapsing infection. Finally, P. falciparum and P. vivax species have different drug resistance patterns in differing geographic regions. For P. falciparum and P. knowlesi infections, the urgent initiation of appropriate therapy is especially critical.

The clinical status of the patient:

Patients diagnosed with malaria are generally categorized as having either uncomplicated or severe malaria. Patients diagnosed with uncomplicated malaria can be effectively treated with oral antimalarials. However, patients who have one or more of the following clinical criteria are considered to have manifestations of more severe disease and should be treated aggressively with parenteral antimalarial therapy: impaired consciousness/coma, severe normocytic anemia [hemoglobin<7], renal failure, acute respiratory distress syndrome, hypotension, disseminated intravascular coagulation, spontaneous bleeding, acidosis, hemoglobinuria, jaundice, repeated generalized convulsions, and/or parasitemia of >5%.

The drug susceptibility of the infecting parasites:

Finally, knowledge of the geographic area where the infection was acquired provides information on the likelihood of drug resistance of the infecting parasite and enables the treating clinician to choose an appropriate drug or drug combination and treatment course. In addition, if a malaria infection occurred despite use of a medicine for chemoprophylaxis, that medicine should not be a part of the treatment regimen. If the diagnosis of malaria is suspected and cannot be confirmed, or if the diagnosis of malaria is confirmed but species determination is not possible, antimalarial treatment effective against chloroquine-resistant P. falciparum must be initiated immediately.

Treatment: Uncomplicated Malaria:

P. falciparum or Species Not Identified – Acquired in Areas without Chloroquine Resistance:

For P. falciparum infections acquired in areas without chloroquine-resistant strains, which include Central America west of the Panama Canal, Haiti, the Dominican Republic, and most of the Middle East, patients can be treated with oral chloroquine. A chloroquine dose of 600 mg base (=1,000 mg salt) should be given initially, followed by 300 mg base (=500 mg salt) at 6, 24, and 48 hours after the initial dose for a total chloroquine dose of 1,500 mg base (=2,500 mg salt). Alternatively, hydroxychloroquine may be used at a dose of 620 mg base (=800 mg salt) by mouth given initially, followed by 310 mg base (=400 mg salt) by mouth at 6, 24, and 48 hours after the initial dose for a total hydroxychloroquine dose of 1,550 mg base (=2,000 mg salt).

P. falciparum or Species Not Identified – Acquired in Areas with Chloroquine Resistance:

For P. falciparum infections acquired in areas with chloroquine resistance, four treatment options are available. The first two treatment options are atovaquone-proguanil (Malarone) or artemether-lumefantrine (Coartem). These are fixed dose combination medicines that can be used for non-pregnant adult and pediatric patients. Both of these options are very efficacious. Quinine sulfate plus doxycycline, tetracycline, or clindamycin is the next treatment option. For the quinine sulfate combination options, quinine sulfate plus either doxycycline or tetracycline is

generally preferred to quinine sulfate plus clindamycin because there are more data on the efficacy of quinine plus doxycycline or tetracycline. Quinine treatment should continue for 7 days for infections acquired in Southeast Asia and for 3 days for infections acquired in Africa or South America. The fourth option, mefloquine, is associated with rare but potentially severe neuropsychiatric reactions when used at treatment doses. We recommend this fourth option only when the other options cannot be used.

For pediatric patients, the treatment options are the same as for adults except the drug dose is adjusted by patient weight. The pediatric dose should never exceed the recommended adult dose. Pediatric dosing may be difficult due to unavailability of noncapsule forms of quinine. If unable to provide pediatric doses of quinine, consider one of the other three options.

If using a quinine-based regimen for children less than eight years old, doxycycline and tetracycline are generally not indicated; therefore, quinine can be given alone for a full 7 days regardless of where the infection was acquired or given in combination with clindamycin as recommended above. In rare instances, doxycycline or tetracycline can be used in combination with quinine in children less than eight years old if other treatment options are not available or are not tolerated, and the benefit of adding doxycycline or tetracycline is judged to outweigh the risk.

If infections initially attributed to "species not identified" are subsequently diagnosed as being due to P. vivax or P. ovale, additional treatment with primaquine or tafenoquine should be administered.

P. malariae and P. knowlesi:

There has been no widespread evidence of chloroquine resistance in P. malariae and P. knowlesi species; therefore, chloroquine (or hydroxychloroquine) may still be used for both of these infections. In addition, any of the regimens listed above for the treatment of chloroquine-resistant malaria may be used for the treatment of P. malariae and P. knowlesi infections.

P. vivax and P. ovale:

Chloroquine (or hydroxychloroquine) remains an effective choice for all P. vivax and P. ovale infections except for P. vivax infections acquired in Papua New Guinea or Indonesia. The regimens listed for the treatment of P. falciparum are also effective and may be used. Reports have confirmed a high prevalence of chloroquine-resistant P. vivax in these two specific areas. Rare cases of chloroquine-resistant P. vivax have also been documented in Burma (Myanmar), India, and Central and South America. Persons acquiring P. vivax infections from regions other than Papua New Guinea or Indonesia should initially be treated with chloroquine. If the patient does not respond to chloroquine, treatment should be changed to one of the two regimens recommended for chloroquine-resistant P. vivax infections, and your state health department and the CDC should be notified (CDC Malaria Hotline: (770) 488-7788 Monday–Friday 9am–5pm EST; (770) 488-7100 after hours, weekends, and holidays).

Persons acquiring P. vivax infections in Papua New Guinea or Indonesia should initially be treated with a regimen recommended for chloroquine-resistant P. vivax infections. The three treatment regimens for chloroquine-resistant P. vivax infections are quinine sulfate plus doxycycline or tetracycline, or, Atovaquone-proguanil, or if other options are not available, mefloquine.

In addition to requiring blood stage treatment, infections with P. vivax and P. ovale can relapse due to hypnozoites that remain dormant in the liver. To eradicate the hypnozoites, patients should be treated with either primaquine phosphate or tafenoquine. Tafenoquine can be used in those 16 years old and over, and is given as a single dose of 300 mg by mouth. If primaquine phosphate is used, CDC recommends a dose of 30 mg (base) by mouth daily for 14 days. Because both primaquine and tafenoquine can cause hemolytic anemia in persons with glucose-6-phosphate-dehydrogenase (G6PD) deficiency, persons must be tested for G6PD deficiency using a quantitative test prior to starting primaquine treatment. For persons with borderline G6PD deficiency or as an alternate to the above regimen, primaquine may be given at the dose of 45 mg (base) by mouth one time per week for 8 weeks; consultation with an expert in infectious disease and/or tropical medicine is advised if this alternative regimen is considered in G6PD-deficient persons. Primaquine and tafenoquine must not be used during pregnancy. Tafenoquine must not be used in children less than 16 years old, or in those with a history of a psychotic disorder.

For pediatric patients greater than 8 years old, the treatment options, with the exception of tafenoquine, are the same as for adults except the drug dose is adjusted by patient weight. The pediatric dose should never exceed the recommended adult dose. For children less than 8 years old, doxycycline and tetracycline are generally not indicated; therefore other treatment options should be used. For pediatric patients <5kg, mefloquine is the only option. If mefloquine is not available or is not being tolerated and if the treatment benefits outweigh the risks, atovaquone-proguanil or artemether-lumefantrine should be used instead. Primaquine should be given to pediatric patients only after they have been screened for G6PD deficiency.

Alternatives for pregnant women:

Malaria infection in pregnant women is associated with high risks of both maternal and perinatal morbidity and mortality. While the mechanism is poorly understood, pregnant women have a reduced immune response and therefore less effectively clear malaria infections. In addition, malaria parasites sequester and replicate in the placenta. Pregnant women are three times more likely to develop severe disease than non-pregnant women acquiring infections from the same area. Malaria infection during pregnancy can lead to miscarriage, premature delivery, low birth weight, congenital infection, and/or perinatal death.

For pregnant women diagnosed with uncomplicated malaria caused by P. malariae, P. vivax, P. ovale, or chloroquine-sensitive P. falciparum infection, prompt treatment with chloroquine (treatment schedule as with non-pregnant adult patients) is recommended. Alternatively, hydroxychloroquine may be given instead. For women in their second or third trimesters,

artemether-lumefantrine is an additional option. For pregnant women diagnosed with uncomplicated malaria caused by chloroquine-resistant P. falciparum infection, women in their second and third trimesters can be treated with artemther lumefantrine, and for all trimesters, mefloquine or a combination of quinine sulfate and clindamycin is recommended. Quinine treatment should continue for 7 days for infections acquired in Southeast Asia and for 3 days for infections acquired elsewhere; clindamycin treatment should continue for 7 days regardless of where the infection was acquired. For pregnant women diagnosed with uncomplicated malaria caused by chloroquine-resistant P. vivax infection, prompt treatment with artemether-lumefantrine (second and third trimesters) or mefloquine (all trimesters) is recommended.

Doxycycline and tetracycline are generally not indicated for use in pregnant women. However, in rare instances, doxycycline or tetracycline can be used in combination with quinine if other treatment options are not available or are not being tolerated, and the benefit of adding doxycycline or tetracycline is judged to outweigh the risks.

According to its U.S. labels, atovaquone/proguanil is not indicated for use in pregnant women because there are no adequate, well-controlled studies in pregnant women. However, for pregnant women diagnosed with uncomplicated malaria caused by chloroquine-resistant P. falciparum infection, atovaquone-proguanil may be used if other treatment options are not available or are not being tolerated, and if the potential benefit is judged to outweigh the potential risks.

For P. vivax or P. ovale infections, primaquine phosphate and tafenoquine for radical treatment of hypnozoites should not be given during pregnancy. Pregnant patients with P. vivax or P. ovale infections should be maintained on chloroquine prophylaxis for the duration of their pregnancy. The chemoprophylactic dose of chloroquine phosphate is 300mg base (=500 mg salt) by mouth once per week. After delivery, pregnant patients with P. vivax or P. ovale infections who do not have G6PD deficiency, subsequent treatment with primaquine phosphate or tafenoquine is needed, but will depend on breastfeeding. If not breastfeeding, either drug can be used. For women who are breastfeeding infants with normal G6PD activity, primaquine phosphate can be given. Tafenoquine is not recommended during breastfeeding.

Treatment: Severe Malaria:

Patients who are considered to have manifestations of more severe disease should be treated aggressively with parenteral antimalarial therapy regardless of the species of malaria seen on the blood smear. If severe malaria is strongly suspected but a laboratory diagnosis cannot be made at that time, blood should be collected for diagnostic testing as soon as it is available and parenteral antimalarial drugs should be started.

All patients with severe malaria, regardless of infecting species, should be treated with intravenous (IV) artesunate. Clinicians caring for patients with suspected severe malaria.

Severe malaria can progress rapidly and must be treated as soon as possible. While timely delivery of IV artesunate is anticipated, health-care providers can consider treating the patient with an oral antimalarial while waiting for IV artesunate to arrive. Health-care providers will

need to decide the most feasible route to administer the drug for patients unable to tolerate an oral antimalarial. For example, if this intolerance is due to nausea and vomiting, an anti-emetic preceding the antimalarial may help. For comatose patients, a nasogastric tube can be considered.

IV artesunate is safe in infants, children, and pregnant women in the second and third trimesters. There are limited clinical data on women taking IV artesunate in the first trimester of pregnancy; no harmful effects have been observed. Given that severe malaria is life threatening for pregnant women and their fetuses, and the lack of other treatment options for severe malaria in the United States, the benefits of treatment with IV artesunate outweigh the risks and IV artesunate should not be withheld. The only contraindication to IV artesunate is known allergy to IV artemisinins.

IV artesunate is well tolerated. While rare, delayed post-artemisinin hemolytic anemia has been noted in published case reports following treatment of severe malaria with IV artesunate in other non-endemic countries. Persons treated for severe malaria with IV artesunate should be monitored for up to 4 weeks after that treatment for evidence of hemolytic anemia. Persons with higher parasitemia seem to have a higher likelihood of delayed hemolytic anemia after treatment with IV artesunate. Depending on the amount of hemolysis, transfusion may be needed [214].

Prevention and control of Malaria

Malaria is a difficult disease to control largely due to the highly adaptable nature of the vector and parasites involved. While effective tools have been and will continue to be developed to combat malaria, inevitably, over time the parasites and mosquitoes will evolve means to circumvent those tools if used in isolation or used ineffectively. To achieve sustainable control over malaria, healthcare professionals will need a combination of new approaches and tools, and research will play a critical role in development of those next-generation strategies.

Special populations:

Malaria has a significant impact on the health of infants, young children, and pregnant women worldwide. More than 800,000 African children under the age of five die of malaria each year. Malaria also contributes to malnutrition in children, which indirectly causes the death of half of all children under the age of five throughout the world. Fifty million pregnant women throughout the world are exposed to malaria each year. In malaria-endemic regions, one-fourth of all cases of severe maternal anemia and 20 percent of all low-birthweight babies are linked to malaria. Scientists are working to better understand how malaria uniquely affects children and pregnant women and to develop new research tools, methods, and products appropriate for these populations.

Vaccine development:

The development of a safe and effective vaccine against malaria will be critical in malaria control, prevention, and eradication efforts. Currently, no licensed vaccine against malaria (or any parasitic disease that afflicts humans) exists. The complexity of the *Plasmodium* parasite and the lack of understanding of critical processes, such as host immune protection and disease pathogenesis, have hampered vaccine development efforts.

Drug development:

Antimalarial drugs, in combination with mosquito control programs, have historically played a key role in controlling malaria in endemic areas, resulting in significant reduction of the geographic range of malarial disease worldwide. Over the years, however, the emergence and spread of drug-resistant parasites has contributed to a reemergence of malaria, turning back the clock on control efforts. The need for new, effective drugs for malaria has become a critical priority on the global malaria research agenda.

Diagnostics:

New and improved diagnostics are essential for the effective control of malaria. Currently, the most reliable technique for diagnosing malaria is, as it was throughout the last century, labor-intensive, relying on highly trained technicians using microscopes to analyze blood smears. Such microscopic analysis is time-consuming, variable in quality, difficult to use in resource-poor field settings, and cannot detect drug resistance. Therefore, NIAID supports research to develop easy-to-use tests that diagnose the malaria parasite causing an infection and identify its drug resistance profile.

Vector management approaches:

Vector management tools such as insecticides, environmental modification, and bed nets have contributed greatly to successful malaria control efforts historically, but have faced setbacks in recent years due to factors such as the emergence of insecticide resistance in mosquitoes. NIAID is supporting research on new vector management strategies to prevent parasite transmission (from humans to mosquitoes and mosquitoes to humans) and reduce the mosquito population [214].

Malaria vaccine

The complicated life cycle of *Plasmodium* presents a challenge to malaria vaccine development. Researchers must determine which life stage of the parasite to target, or whether the vaccine needs to combine elements that target more than one life stage. However, recent findings allow us to be optimistic about the possibility of an effective malaria vaccine.

Malaria is a bit different from many of the diseases we currently vaccinate for because it does not confer so-called sterile immunity. This means that if you become ill from malaria and recover, you can be infected over and over again. The fact that your immune system responded to malaria in the past will not prevent future infection. This is very different from a disease such as measles: most people who contract measles will be immune to future measles infection for life. With malaria, there is some evidence of a *degree* of naturally acquired immunity – someone who has had malaria in the past can still get it again, but she will probably get a less severe case. In many African countries where malaria is common, most people who are re-infected with malaria experience only mild symptoms due to this partial acquired immunity. This is also the reason that malaria is so deadly for children under five. These children have not yet acquired any level of immunity to the parasite, and they are much more likely to experience a severe case that may lead to fatal complications. Moreover, this is also the reason that foreigners who have never experienced malaria must be very careful – they may develop a very serious case when they first are infected. Finally, naturally acquired partial immunity does not last long. In fact, when someone has lived in Africa his entire life and leaves for even a year, he will lose this partial immunity and once again be as vulnerable to malaria as someone who had never been infected [215]. So, one approach to developing a malaria vaccine would be to understand the mechanism of partial immunity and develop a vaccine based on that principle.

Another avenue that has given direction to malaria vaccine researchers is the concept of immunizing with a live attenuated (weakened) whole parasite in its sporozoite form. Support for this idea came in a 1967 study in which Nussenzweig et al. immunized mice with radiation-

attenuated *Plasmodium berghei* (a non-human form of malaria) sporozoites and saw that the mice were protected in a later challenge with infectious sporozoites [216].

Adapting this idea to humans in 2002, Hoffman et al. showed that they could use gamma radiation to attenuate the sporozoites inside infected Anopheles mosquitoes, and thus almost completely protect humans. Human subjects were exposed to the bites of infected mosquitoes, which injected the irradiated sporozoites into the subjects. The sporozoites could travel into the liver cells, but could mature no further. These weakened sporozoites were still able to elicit an immune response in the human host, but because they could not develop further than the liver, the host would not get sick. As a result, the next time an infected mosquito took a blood meal from the immunized person and injected the person with *Plasmodium* sporozoites, the immune system would recognize the threat and eliminate the parasite before it caused disease.

This irradiation approach had two major flaws: it was not cost effective and not practical on a large scale [217].

Recent research:

Scientists have expanded on what was learned in the 2002 study to develop many potential malaria vaccines. Instead of attempting a live attenuated vaccine, most scientists today are using technologies to isolate and deliver specific antigens in a vaccine [218]. And because the parasite has three different life stages, there are three distinct vaccines approaches being investigated.

Pre-erythrocytic vaccines:

Target the infectious phase and aim either to prevent the sporozoites from getting into the liver cells or to destroy infected liver cells [219].The most significant challenge for a pre-erythrocytic vaccine is the time frame: sporozoites reach the liver less than an hour after being injected by the mosquito. As a result, the immune system has a limited amount of time to eliminate the parasite. Although most of the potential pre-erythrocytic vaccines are still in Phase I or Phase II trials, one vaccine is currently in Phase III trials and is showing promise: the RTS,S vaccine. (Note that Phase I studies evaluate for safety, Phase II tests evaluate dosing, and Phase III tests assess overall efficacy [220].

In order to develop the RTS,S vaccine, developers identified the protein that was most responsible for protection in the irradiated sporozoite trial from 2002. This antigen is known as the circumsporozoite protein, or CS protein. Although this antigen is protective, it is not very

immunogenic on its own, meaning that it is not good at stimulating an immune response. Thus, scientists fused the Hepatitis B surface antigen (the antigen responsible for providing protection in the Hepatitis B vaccine) with an antigen from the CS protein. In order to stimulate the immune system even further, scientists employed a compound called an adjuvant that boosts the immune system's response to the antigen. The goal is to induce high levels of antibodies to both block the sporozoites from entering the liver cells and to tag specific infected cells for destruction.

Erythrocytic vaccines or blood-stage vaccines:

Aim to stop the rapid invasion and asexual reproduction of the parasite in the red blood cells. Recall that the blood stage is the time when symptoms appear and is also the most destructive to the patient due to the bursting of red blood cells. Because of the huge number of merozoites produced during this stage – 40,000 merozoites are released for each infected liver cell – a blood-stage vaccine can aim only to reduce the number of merozoites infecting red blood cells rather than completely block their replication [221].Currently there are no blood-stage vaccines that have had the success of the RTS,S vaccine and most are still undergoing Phase I or II trials.

Finally, another type of vaccine targets the stage of sexual reproduction that occurs in the mosquito gut. This approach is known as a **transmission blocking vaccine (TBV)** because it aims to kill the vector, the Anopheles mosquito, to stop further spread of the parasite. This is an indirect approach to a vaccine because it will not directly protect an individual who gets the parasite but rather will stop the continued spread [222].

References:

1. Walsch K.C., Understanding Malaria and Lyme Disease., USA, 2010, pp. 87
2. Celli A: A History of Malaria in the Italian Campagna from Ancient Times.. London 1933.
3. Stephens JWW: Blackwater Fever, A Historical Survey and Summary made over a Century London: Hodder and Stoughton 1937.
4. Scott HH: A History of Tropical Medicine London: Edward Arnold 1939, 1.
5. Russell PF: Man's Mastery of Malaria London: Oxford University Press 1965.
6. Foster WD: A History of Parasitology Edinburgh: Livingstone 1965.
7. Garnham PCC: Malaria Parasites and other Haemosporidia Oxford: Blackwell Scientific Publications 1966.
8. Garnham PCC: Progress in Parasitology London: Athlone Press 1971.
9. Harrison G: Mosquitoes and Malaria: A History of the Hostilities since 1880 London: John Murray 1978.
10. Bruce-Chwatt LJ: History of malaria from prehistory to eradication. Malaria: Principles and Practice of Malariology Edinburgh: Churchill LivingstoneWernsdorfer WH, McGregor I 1988, 1:1-59.
11. Desowitz R: The Malaria Capers New York: WW Norton 1991.
12. McGregor I: Malaria. The Wellcome Trust Illustrated History of Tropical Diseases London: The Wellcome TrustCox FEG 1996, 230-247.
13. Poser CM, Bruyn GW: An Illustrated History of Malaria New York: Parthenon 1999.
14. Schlagenhauf P: Malaria from pre-history to present. Infect Dis Clin N Amer 2004, 18:189-205
15. Petrov P., Malaria. MF, Sofia, Bulgaria, 1990, 125 pp. (in BG)
16. Cheesbrough Monica (2006). District laboratory practice in tropical countries. New York-Cambridge press. Part1, 2nd edition Pp249-258.
17. Arora DR and Arora B (2009). Medical Parasitology 2nd edition.Pp.67-81.
18. Gilles HM,Fletcher KA,Hendricks RG(2007). Glucose-6-phosphate dehydrogenase deficiency, sickling and malaria in African children in South Western Nigeria. Lancet 1:138-140.
19. World Malaria Report. 2011. http://www.who.int/malaria/world_malaria_report_2011/9789241564403_eng.pdf.
20. Hay SI, Guerra CA, Tatem AJ, Noor AM, Neve Rw. The global distribution and population at risk of malaria: past, present, and future. Lancet Infect Dis. 2004;4(6):327–336. doi: 10.1016/S1473-3099(04)01043-6.
21. Sullivan D. Uncertainty in Mapping Malaria Epidemiology: Implications for Control. Epidemiol Rev. 2010;1:175–187. doi: 10.1093/epirev/mxq013.
22. Malaria endemicity. http://www.map.ox.ac.uk/explore/about-malaria/malaria-endemicity/

23. Hay SI, Smitha DL, Snow RW. Measuring malaria endemicity from intense to interrupted transmission. Lancet Infect Dis. 2008;8(6):369–378. doi: 10.1016/S1473-3099(08)70069-0.
24. Kleinschmidt I, Bagayoko M, Clarke GPY, Craig M, Le Sueur D. A spatial statistical approach to malaria mapping. Int J Epidemiol. 2000;29:355–361. doi: 10.1093/ije/29.2.355.
25. Kleinschmidt I, Omumbo J, Briet O, van de Giesen N, Sogoba N, Mensah NK, Windmeijer P, Moussa M, Teuscher T. An empirical malaria distribution map for West Africa. Trop Med Int Health. 2001;6:779–786. doi: 10.1046/j.1365-3156.2001.00790.x.
26. Rogers DJ, Randolph SE, Snow RW, Hay SI. Satellite imagery in the study and forecast of malaria. Nature. 2002;415:710–715. doi: 10.1038/415710a.
27. Omumbo JA, Hay SI, Snow RW, Tatem AJ, Rogers DJ. Modelling malaria risk in East Africa at high-spatial resolution. Trop Med Int Health. 2005;10:557–566. doi: 10.1111/j.1365-3156.2005.01424.x.
28. Rogers DJ. Models for vectors and vector-borne diseases. Adv Parasitol. 2006;62:1–35. doi: 10.1016/S0065-308X(05)62001-5.
29. Gemperli A, Vounatsou P, Kleinschmidt I, Bagayoko M, Lengeler C, Smith C. Spatial patterns of infant mortality in Mali: the effect of malaria endemicity. Am J Epidemiol. 2004;159:64–72. doi: 10.1093/aje/kwh001.
30. Gemperli A, Vounatsou P, Sogoba N, Smith T. Malaria mapping using transmission models: application to survey data from Mali. Am J Epidemiol. 2006;163:289–297. doi: 10.1093/aje/kwj026.
31. Rattanasiri S, Bohning D, Rojanavipart P, Athipanyakom S. A mixture model application in disease mapping of malaria. Southeast Asian J Trop Med Public Health. 2004;35:38–47.
32. Gosoniu L, Vounatsou P, Sogoba N, Smith T. Bayesian modelling of geostatistical malaria risk data. Geospat Health. 2006;1:127–139.
33. Noor AM, Clements ACA, Gething PW, Moloney G, Borle M, Shewchuk T, Fieno SI, Neve RW. Spatial prediction of Plasmodium falciparum prevalence in Somalia. Malar J. 2008;7:159. doi: 10.1186/1475-2875-7-159.
34. Hay SI, Guerra CA, Gething PW, Patil AP, Tatem AJ, Noor AM, Kabaria CW, Manh BH, Elyazar IR, Brooker S, Smith DL, Moyeed RA, Snow RW. A world malaria map: Plasmodium falciparum endemicity in 2007. PLoS Med. 2009;6(3):e1000048. doi: 10.1371/journal.pmed.1000048.
35. Guerra CA. Mapping the contemporary global distribution limits of malaria using empirical data and expert opinion. Oxford: University of Oxford; 2007. p. 258.
36. Guerra CA, Gikandi PW, Tatem AJ, Noor AM, Smith DL, Hay SI, Snow RW. The limits and intensity of Plasmodium falciparum transmission: implications for malaria control and elimination worldwide. PLoS Medicine. 2008;5(2):e38. doi: 10.1371/journal.pmed.0050038.

37. Guerra CA, Howes RE, Patil AP, Gething PW, Van Boeckel TP, Temperley WH, Kabaria CW, Tatem AJ, Manh BH, Elyazar IR, Baird JK, Snow RW, Hay SI. The International Limits and Population at Risk of Plasmodium vivax Transmission in 2009. PLoS Negl Trop Dis. 2010;4(8):e774. doi: 10.1371/journal.pntd.0000774.
38. Macdonald G. Local features of malaria. Oxford University Press; London: 1957. The epidemiology and control of malaria; pp. 63–99.
39. Malaria recommendation update: Greece. http://www.cdc.gov/malaria/malariagreece.htm.
40. European Centre for Disease Prevention and Control. RISK ASSESSMENT - UPDATE. European Centre for Disease Prevention and Control; Stockholm: 2011. Autochthonous Plasmodium vivax malaria in Greece.
41. Culleton RL, Mita T, Ndounga M, Unger H, Cravo PVL, Paganotti GM, Takahashi N, Kaneko A, Eto H, Tinto H, Karema C, D'Alessandro U, Do Rosário V, Kobayakawa T, Ntoumi F, Carter R, Tanabe K. Failure to detect Plasmodium vivax in West and Central Africa by PCR species typing. Malar J. 2008;7:174. doi: 10.1186/1475-2875-7-174.
42. Pullan RL, Bukirwa H, Staedke SG, Snow RW, Brooker S. Plasmodium infection and its risk factors in eastern Uganda. Malar J. 2010;9:2. doi: 10.1186/1475-2875-9-2.
43. Iriemenam NC, Dosunmu AO, Oyibo WA, Fagbenro-Beyioku AF. Knowledge, attitude, perception of malaria and evaluation of malaria parasitaemia among pregnant women attending antenatal care clinic in metropolitan Lagos, Nigeria. J Vector Borne Dis. 2011;48:12–17.
44. Das A, Anvikar AR, Cator LJ, Dhiman RC, Eapen A, Mishra NB, Nagpal N, Nanda N, Raghavendra K, Read AF, Sharma SK, Singh OP, Singh V, Sinni P, Srivastava HC, Sullivan SA, Sutton PL, Thomas MB, Carlton JM, Valecha N. Malaria in India: The Center for the Study of Complex Malaria in India. Acta Trop. 2011;28.
45. Dhangadamajhi G, Kar SK, Ranjit MR. High prevalence and gender bias in distribution of Plasmodium malariae infection in central east-coast India. Trop Biomed. 2009;26(3):326–333.
46. Fuehrer H, Starzengruber P, Swoboda P, Khan WA, Matt J, Ley B, Thriemer K, Haque R, Yunus EB, Hossain SM, Walochnik J, Noedl H. Indigenous Plasmodium ovale Malaria in Bangladesh. Am J Trop Med Hyg. 2010;83(1):75–78. doi: 10.4269/ajtmh.2010.09-0796.
47. Steenkeste N, Rogers WO, Okell L, Jeanne I, Incardona S, Duval L, Chy S, Hewitt S, Chou M, Socheat D, Babin F, Ariey F, Rogier C. Sub-microscopic malaria cases and mixed malaria infection in a remote area of high malaria endemicity in Rattanakiri province, Cambodia: implication for malaria elimination. Mal J. 2010;9:108. doi: 10.1186/1475-2875-9-108.
48. Putaporntip C, Hongsrimuang T, Seethamchai S, Kobasa T, Limkittikul K, Cui L, Jongwutiwes S. Differential prevalence of Plasmodium infections and cryptic

Plasmodium knowlesi malaria in humans in Thailand. J Infect Dis. 2009; 199:1143–50. doi: 10.1086/597414.
49. Jiang N, Chang Q, Sun X, Lu H, Zhang J, Wahlgren M, Chen Q. Co-infections with Plasmodium knowlesi and other malaria parasites, Myanmar. Emerg Infect Dis. 2010;16(9):1476–1478. doi: 10.3201/eid1609.100339.
50. Arevalo-Herrera ML, Quinones M, Guerra C, Céspedes N, Giron S, Ahumada M, Pineros JG, Padilla N, Terrientes Z, Rosas A, Padilla JC, Escalante A, Beier JC, Herrera S. Malaria in selected non-Amazonian countries of Latin America. Acta Trop. 2011;121(3):303–14. doi: 10.1016/j.actatropica.2011.06.008.
51. Culleton R, Ndounga M, Yildiz Zeyrek F, Coban C, Casimiro PN, Takeo S, Tsuboi T, Yadava A, Carter R, Tanabe K. Evidence for the transmission of Plasmodium vivax in the Republic of the Congo, West Central Africa. J Infect Dis. 2009;200:1465–9. doi: 10.1086/644510.
52. Ryan J, Stoute JA, Amon J, Dunton RF, Mtalib R, Koros J, Owour B, Luckhart S, Wirtz RA, Barnwell JW, Rosenberg R. Evidence for transmission of Plasmodium vivax among a Duffy antigen negative population in Western Kenya. Am J Trop Med Hyg. 2006; 75(4):575–581.
53. Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM. Vivax malaria: neglected and not benign. Am J Trop Med Hyg. 2007;77(6):79–87.
54. Dash AP, Valecha N, Anvikar AR, Kumar A. Malaria in India: challenges and opportunities. J Biosci. 2008;33(4):583–592. doi: 10.1007/s12038-008-0076-x.
55. Beljaev AE. The malaria situation in the WHO eastern Mediterranean region. Med Parazitol (Mosk) 2000; 2:12–15.
56. Brooker S, Leslie T, Kolaczinski K, Mohsen E, Mehboob N, Saleheen S, Khudonazarov J, Freeman T, Clements A, Rowland M, Kolaczinski J. Spatial Epidemiology of Plasmodium vivax, Afghanistan. Emerg Infect Dis. 2006;12(10):1600–1602. doi: 10.3201/eid1210.060051.
57. Zakeri S, Najafabadi ST, Zare A, Djadid ND. Detection of malaria parasites by nested PCR in south-eastern, Iran: evidence of highly mixed infections in Chahbahar district. Malar J. 2002; 1:2. doi: 10.1186/1475-2875-1-2.
58. Celikbas AK, Ergönül O, Baykam N, Eren S, Güven T, Dokuzoguz B. Malaria in Turkey and 14 years of clinical experience. Mikrobiyol Bul. 2006; 40(3):237–43.
59. Oliveira-Ferreira J, Lacerda MVG, Brasil P, Ladislau JLB, Tauil PL, Tadeu Daniel-Ribeiro C. Malaria in Brazil: an overview. Malar J. 2010;9:115. doi: 10.1186/1475-2875-9-115.
60. Padilla Rodríguez JC, Aacute;lvarez Uribe G, Montoya Araújo R, Chaparro Narváez P, Herrera Valencia S. Epidemiology and control of malaria in Colombia. Mem Inst Oswaldo Cruz. 2011; 106:114–122.
61. Manock SR, Jacobsen KH, de Bravo NB, Russell KL, Negrete M, Olson JG, Sanchez JLP, Blair J, Smalligan RD, Quist BK, Espín JF, Espinoza WR, MacCormick F, Fleming

LC, Kochel T. Etiology of Acute Undifferentiated Febrile Illness in the Amazon Basin of Ecuador. Am J Trop Med Hyg. 2009;81(1):146–151.
62. Collins WE, Jeffery GM. Plasmodium ovale: parasite and disease. Clin Microbiol Rev. 2005; 18(3):570–581. doi: 10.1128/CMR.18.3.570-581.2005.
63. Macedo de Oliveira A, Mutemba R, Morgan J, Streat E, Roberts J, Menon M, Mabunda S. Prevalence of malaria among patients attending Public Health facilities in Maputo city, Mozambique. Am J Trop Med Hyg. 2011;85(6):1002–7. doi: 10.4269/ajtmh.2011.11-0365.
64. Taylor SM, Messina JP, Hand CC, Juliano JJ, Uwonga J, Tshefu AK, Atua B, Emch M, Meshnick SR. Molecular malaria epidemiology: mapping and burden estimates for the Democratic Republic of the Congo, 2007. PLoS ONE. 2011;6(1):e16420.
65. Oguike MC, Betson M, Burke M, Nolder D, Russell Stothard J, Kleinschmidt I, Proietti C, Bousema T, Ndounga M, Tanabe K, Ntege E, Cullettan R, Sutherland CJ. Plasmodium ovale curtisi and Plasmodium ovale wallikeri circulate simultaneously in African communities. Int J Parasitol. 2011;41(6–10):677–683.
66. Win TT, Lin K, Mizuno S, Zhou M, Liu Q, Ferreira MU, Tantular IS, Kojima S, Ishii A, Kawamoto F. Wide distribution of Plasmodium ovale in Myanmar. Trop Med Int Health. 2002;7(3):231–239. doi: 10.1046/j.1365-3156.2002.00857.x
67. Marathe Date V, Shah HN, Tripathi JR. Plasmodium ovale – a case report from Gujarat. J Vect Borne Dis. 2006;43:206–208.
68. Lim YA, Mahmud R, Hoong Chew C, Heng Chua TTK. Plasmodium ovale infection in Malaysia: first imported case. Malar J. 2010;9:272. doi: 10.1186/1475-2875-9-272.
69. Wickremasinghe R, Galapaththy GNL, Fernando WAP, de Monbrison F, Wijesinghe RS, Mendis KN, Picot S, Ringwald P, Wickremasinghe AR. Short report: an indigenous case of Plasmodium ovale infection in Sri Lanka. Am J Trop Med Hyg. 2008;78(2):206–207.
70. Siswantoro H, Russell B, Ratcliff A, Prasetyorini B, Chalfein F, Marfurt J, Kenangalem E, Wuwung M, Piera KA, Ebsworth EP, Anstey NM, Tjitra E, Price RN. In vivo and in vitro efficacy of cloroquine against Plasmodium malariae and P. ovale in Papua, Indonesia. Antimicrob Agents Chemother. 2011;55(1):197–202. doi: 10.1128/AAC.01122-10.
71. Cabrera BD, Arambulo PV. Malaria in the Republic of the Philippines. A review. Acta Trop. 1977;34(3):265–79.
72. Collins WE, Jeffery GM. Plasmodium malariae: parasite and disease. Clin Microbiol Rev. 2007;20(4):579–592. doi: 10.1128/CMR.00027-07
73. Rahman W, Chotivanich K, Silamut K, Tanomsing N, Hossain A, Faiz MA, Dondorp AM, Maude RJ. Plasmodium malariae in Bangladesh. Trans R Soc Trop Med Hyg. 2010;104(1):78–80. doi: 10.1016/j.trstmh.2009.06.014.
74. Rosanas-Urgell A, Mueller D, Betuela I, Barnadas C, Iga J, Zimmerman PA, del Portillo HA, Siba P, Mueller I, Felger I. Comparison of diagnostic methods for the detection and

quantification of the four sympatric Plasmodium species in field samples from Papua New Guinea. Malar J. 2010;9:361. doi: 10.1186/1475-2875-9-361.
75. Lee KS, Cox-Singh J, Brooke G, Matusop A, Singh B. Plasmodium knowlesi from archival blood films: further evidence that human infections are widely distributed and not newly emergent in Malaysian Borneo. Int J Parasitol. 2009;39:1125–28. doi: 10.1016/j.ijpara.2009.03.003.
76. Jooven-Neoh WF, Lung Chong K, Vui Ling Wong CM, Ying Lau T. Incidence of malaria in the interior division of Sabah, Malaysian Borneo, based on Nested PCR. J Parasitol Res. 2011;2011:104284.
77. Luchavez J, Espino F, Curameng P, Espina R, Bell D, Chiodini P, Nolder D, Sutherland C, Lee K, Singh B. Human infection with Plasmodium knowlesi, the Philippines. Emerg Infect Dis. 2008;14(5):811–813. doi: 10.3201/eid1405.071407.
78. Jeslyn WPS, Huat TC, Vernoon L, Zhi Irene LM, Kim Sung L, Piao Jarrod L, Singh B, Ching NL. Molecular epidemiological investigation of Plasmodium knowlesi in humans and macaques in Singapore. Vector Borne and Zoonotic Dis. 2011;11(2):131–135. doi: 10.1089/vbz.2010.0024.
79. Khim N, Siv S, Kim S, Mueller T, Fleischmann E, Singh B, Divis PCS, Steenkeste N, Duval L, Bouchier C, Duong S, Ariey F, Ménard D. Plasmodium knowlesi infection in humans, Cambodia 2007–2010. Emerg Infect Dis. 2011;17(10):1900–1902. doi: 10.3201/eid1710.110355.
80. Van den Eede P, Van HN, Overmeir C, Vythilingam I, Ngo Duc T, Hung LX, Manh HN, Anné J, D'Alessandro U, Erhart A. Human Plasmodium Knowlesi infections in young children in central Vietnam. Malar J. 2009;8:249. doi: 10.1186/1475-2875-8-249.
81. https://www.cdc.gov/malaria/about/biology/
82. Wykes MN, Good MF. What have we learnt from mouse models for the study of malaria? Eur J Immunol. 2009;39:2004–7.
83. Sauerwein RW, Roestenberg M, Moorthy VS. Experimental human challenge infections can accelerate clinical malaria vaccine development. Nat Rev Immunol. 2011;11:57–64.
84. Engwerda CR, Minigo G, Amante FH, McCarthy JS. Experimentally induced blood stage malaria infection as a tool for clinical research. Trends Parasitol. 2012;28:515–21.
85. Tran TM, Samal B, Kirkness E, Crompton PD. Systems immunology of human malaria. Trends Parasitol. 2012;28:248–57.
86. Offeddu V, Thathy V, Marsh K, Matuschewski K. Naturally acquired immune responses against Plasmodium falciparum sporozoites and liver infection. Int J Parasitol. 2012; 42:535–48.
87. Tran TM, Li S, Doumbo S, Doumtabe D, Huang CY, Dia S, Bathily A, Sangala J, Kone Y, Traore A, Niangaly M, Dara C, Kayentao K, Ongoiba A, Doumbo OK, Traore B, Crompton PD. An Intensive Longitudinal Cohort Study of Malian Children and Adults Reveals No Evidence of Acquired Immunity to Plasmodium falciparum Infection. Clin Infect Dis. 2013; 57:40–7.

88. Honda T, Miyachi Y, Kabashima K. Regulatory T cells in cutaneous immune responses. J Dermatol Sci. 2011;63:75–82.
89. Crispe IN. The liver as a lymphoid organ. Annu Rev Immunol. 2009; 27:147–63.
90. Guilbride DL, Gawlinski P, Guilbride PD. Why functional pre-erythrocytic and bloodstage malaria vaccines fail: a meta-analysis of fully protective immunizations and novel immunological model. PLoS One. 2010; 5:e10685.
91. da Silva HB, Caetano SS, Monteiro I, Gomez-Conde I, Hanson K, Penha-Goncalves C, Olivieri DN, Mota MM, Marinho CR, D'Imperio Lima MR, Tadokoro CE. Early skin immunological disturbance after Plasmodium-infected mosquito bites. Cell Immunol. 2012; 277:22–32.
92. Good MF, Doolan DL. Malaria vaccine design: immunological considerations. Immunity. 2010; 33:555–66.
93. Usynin I, Klotz C, Frevert U. Malaria circumsporozoite protein inhibits the respiratory burst in Kupffer cells. Cell Microbiol. 2007; 9:2610–28.
94. Nussenzweig RS, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghei. Nature. 1967; 216:160–2.
95. Gwadz RW, Cochrane AH, Nussenzweig V, Nussenzweig RS. Preliminary studies on vaccination of rhesus monkeys with irradiated sporozoites of Plasmodium knowlesi and characterization of surface antigens of these parasites. Bull World Health Organ. 1979; 57(Suppl 1):165–73.
96. Hoffman SL, Goh LM, Luke TC, Schneider I, Le TP, Doolan DL, Sacci J, de la Vega P, Dowler M, Paul C, Gordon DM, Stoute JA, Church LW, Sedegah M, Heppner DG, Ballou WR, Richie TL. Protection of humans against malaria by immunization with radiation-attenuated Plasmodium falciparum sporozoites. J Infect Dis. 2002; 185:1155–64.
97. Vaughan AM, Wang R, Kappe SH. Genetically engineered, attenuated whole-cell vaccine approaches for malaria. Hum Vaccin. 2010; 6:107–13.
98. Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, van Gemert GJ, van de Vegte-Bolmer M, van Schaijk B, Teelen K, Arens T, Spaarman L, de Mast Q, Roeffen W, Snounou G, Renia L, van der Ven A, Hermsen CC, Sauerwein R. Protection against a malaria challenge by sporozoite inoculation. N Engl J Med. 2009; 361:468–77.
99. Epstein JE, Tewari K, Lyke KE, Sim BK, Billingsley PF, Laurens MB, Gunasekera A, Chakravarty S, James ER, Sedegah M, Richman A, Velmurugan S, Reyes S, Li M, Tucker K, Ahumada A, Ruben AJ, Li T, Stafford R, Eappen AG, Tamminga C, Bennett JW, Ockenhouse CF, Murphy JR, Komisar J, Thomas N, Loyevsky M, Birkett A, Plowe CV, Loucq C, Edelman R, Richie TL, Seder RA, Hoffman SL. Live attenuated malaria vaccine designed to protect through hepatic CD8(+) T cell immunity. Science. 2011; 334:475–80.

100. Trieu A, Kayala MA, Burk C, Molina DM, Freilich DA, Richie TL, Baldi P, Felgner PL, Doolan DL. Sterile protective immunity to malaria is associated with a panel of novel P. falciparum antigens. Mol Cell Proteomics. 2011; 10 M111 007948.
101. Krzych U, Lyon JA, Jareed T, Schneider I, Hollingdale MR, Gordon DM, Ballou WR. T lymphocytes from volunteers immunized with irradiated Plasmodium falciparum sporozoites recognize liver and blood stage malaria antigens. J Immunol. 1995; 155:4072–7.
102. Doolan DL, Southwood S, Freilich DA, Sidney J, Graber NL, Shatney L, Bebris L, Florens L, Dobano C, Witney AA, Appella E, Hoffman SL, Yates JR 3rd, Carucci DJ, Sette A. Identification of Plasmodium falciparum antigens by antigenic analysis of genomic and proteomic data. Proc Natl Acad Sci U S A. 2003; 100:9952–7.
103. Roestenberg M, Teirlinck AC, McCall MB, Teelen K, Makamdop KN, Wiersma J, Arens T, Beckers P, van Gemert G, van de Vegte-Bolmer M, van der Ven AJ, Luty AJ, Hermsen CC, Sauerwein RW. Long-term protection against malaria after experimental sporozoite inoculation: an open-label follow-up study. Lancet. 2011; 377:1770–6.
104. Bijker EM, Bastiaens GJ, Teirlinck AC, van Gemert GJ, Graumans W, van de Vegte- Bolmer M, Siebelink-Stoter R, Arens T, Teelen K, Nahrendorf W, Remarque EJ, Roeffen W, Jansens A, Zimmerman D, Vos M, van Schaijk BC, Wiersma J, van der Ven AJ, de Mast Q, van Lieshout L, Verweij JJ, Hermsen CC, Scholzen A, Sauerwein RW. Protection against malaria after immunization by chloroquine prophylaxis and sporozoites is mediated by preerythrocytic immunity. Proc Natl Acad Sci U S A. 2013; 110:7862–7.
105. Miller LH, Ackerman HC, Su XZ, Wellems TE. Malaria biology and disease pathogenesis: insights for new treatments. Nat Med. 2013; 19:156–67.
106. Kraemer SM, Smith JD. A family affair: var genes, PfEMP1 binding, and malaria disease. Curr Opin Microbiol. 2006; 9:374–80.
107. Turner L, Lavstsen T, Berger SS, Wang CW, Petersen JE, Avril M, Brazier AJ, Freeth J, Jespersen JS, Nielsen MA, Magistrado P, Lusingu J, Smith JD, Higgins MK, Theander TG. Severe malaria is associated with parasite binding to endothelial protein C receptor. Nature. 2013; 498:502–5.
108. Clark IA, Alleva LM, Mills AC, Cowden WB. Pathogenesis of malaria and clinically similar conditions. Clin Microbiol Rev. 2004; 17:509–39. table of contents.
109. Hunt NH, Grau GE. Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. Trends Immunol. 2003; 24:491–9.
110. White NJ, Turner GD, Day NP, Dondorp AM. Lethal malaria: marchiafava and bignami were right. J Infect Dis. 2013; 208:192–8.
111. Day NP, Hien TT, Schollaardt T, Loc PP, Chuong LV, Chau TT, Mai NT, Phu NH, Sinh DX, White NJ, Ho M. The prognostic and pathophysiologic role of pro- and antiinflammatory cytokines in severe malaria. J Infect Dis. 1999; 180:1288–97.

112. Lyke KE, Burges R, Cissoko Y, Sangare L, Dao M, Diarra I, Kone A, Harley R, Plowe CV, Doumbo OK, Sztein MB. Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe Plasmodium falciparum malaria and matched uncomplicated malaria or healthy controls. Infect Immun. 2004; 72:5630–7.
113. Walther M, Woodruff J, Edele F, Jeffries D, Tongren JE, King E, Andrews L, Bejon P, Gilbert SC, De Souza JB, Sinden R, Hill AV, Riley EM. Innate immune responses to human malaria: heterogeneous cytokine responses to blood-stage Plasmodium falciparum correlate with parasitological and clinical outcomes. J Immunol. 2006; 177:5736–45.
114. Claessens A, Adams Y, Ghumra A, Lindergard G, Buchan CC, Andisi C, Bull PC, Mok S, Gupta AP, Wang CW, Turner L, Arman M, Raza A, Bozdech Z, Rowe JA. A subset of group A-like var genes encodes the malaria parasite ligands for binding to human brain endothelial cells. Proc Natl Acad Sci U S A. 2012; 109:E1772–81.
115. Kurtzhals JA, Adabayeri V, Goka BQ, Akanmori BD, Oliver-Commey JO, Nkrumah FK, Behr C, Hviid L. Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria. Lancet. 1998; 351:1768–72.
116. Taylor SM, Parobek CM, Fairhurst RM. Haemoglobinopathies and the clinical epidemiology of malaria: a systematic review and meta-analysis. The Lancet infectious diseases. 2012; 12:457–68.
117. Turner L, Lavstsen T, Berger SS, Wang CW, Petersen JE, Avril M, Brazier AJ, Freeth J, Jespersen JS, Nielsen MA, Magistrado P, Lusingu J, Smith JD, Higgins MK, Theander TG. Severe malaria is associated with parasite binding to endothelial protein C receptor. Nature. 2013; 498:502–5.
118. Waisberg M, Cerqueira GC, Yager SB, Francischetti IM, Lu J, Gera N, Srinivasan P, Miura K, Rada B, Lukszo J, Barbian KD, Leto TL, Porcella SF, Narum DL, El-Sayed N, Miller LH, Pierce SK. Plasmodium falciparum merozoite surface protein 1 blocks the proinflammatory protein S100P. Proc Natl Acad Sci U S A. 2012; 109:5429–34.
119. Stevenson MM, Riley EM. Innate immunity to malaria. Nat Rev Immunol. 2004; 4:169–80.
120. Guermonprez P, Helft J, Claser C, Deroubaix S, Karanje H, Gazumyan A, Darasse-Jeze G, Telerman SB, Breton G, Schreiber HA, Frias-Staheli N, Billerbeck E, Dorner M, Rice CM, Ploss A, Klein F, Swiecki M, Colonna M, Kamphorst AO, Meredith M, Niec R, Takacs C, Mikhail F, Hari A, Bosque D, Eisenreich T, Merad M, Shi Y, Ginhoux F, Renia L, Urban BC, Nussenzweig MC. Inflammatory Flt3l is essential to mobilize dendritic cells and for T cell responses during Plasmodium infection. Nat Med. 2013; 19:730–8.
121. Porcherie A, Mathieu C, Peronet R, Schneider E, Claver J, Commere PH, Kiefer-Biasizzo H, Karasuyama H, Milon G, Dy M, Kinet JP, Louis J, Blank U, Mecheri S.

Critical role of the neutrophil-associated high-affinity receptor for IgE in the pathogenesis of experimental cerebral malaria. J Exp Med. 2011; 208:2225–36.
122. Hansen DS, D'Ombrain MC, Schofield L. The role of leukocytes bearing Natural Killer Complex receptors and Killer Immunoglobulin-like Receptors in the immunology of malaria. Curr Opin Immunol. 2007; 19:416–23.
123. Horowitz A, Newman KC, Evans JH, Korbel DS, Davis DM, Riley EM. Cross-talk between T cells and NK cells generates rapid effector responses to Plasmodium falciparum-infected erythrocytes. J Immunol. 2010; 184:6043–52.
124. Schofield L, Mueller I. Clinical immunity to malaria. Current molecular medicine. 2006; 6:205–21.
125. Grau GE, Taylor TE, Molyneux ME, Wirima JJ, Vassalli P, Hommel M, Lambert PH. Tumor necrosis factor and disease severity in children with falciparum malaria. N Engl J Med. 1989; 320:1586–91.
126. Molineaux, L. The impact of parasitic diseases and their control on mortality, with emphasis on malaria and Africa. In: Vallin, J.; Lopez, A., editors. Health policy, social policy, and mortality prospects. Liege: Ordina Editions; 1985. p. 13-44.
127. Marsh K, Kinyanjui S. Immune effector mechanisms in malaria. Parasite Immunol. 2006; 28:51–60.
128. Sinton JA. Immunity or Tolerance in Malarial Infections: (Section of Comparative Medicine). Proc R Soc Med. 1938; 31:1298–302.
129. Good-Jacobson KL, Shlomchik MJ. Plasticity and heterogeneity in the generation of memory B cells and long-lived plasma cells: the influence of germinal center interactions and dynamics. J Immunol. 2010; 185:3117–25.
130. Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med. 2007; 357:1903–15.
131. Donati D, Mok B, Chene A, Xu H, Thangarajh M, Glas R, Chen Q, Wahlgren M, Bejarano MT. Increased B cell survival and preferential activation of the memory compartment by a malaria polyclonal B cell activator. J Immunol. 2006; 177:3035–44.
132. Crompton PD, Mircetic M, Weiss G, Baughman A, Huang CY, Topham DJ, Treanor JJ, Sanz I, Lee FE, Durbin AP, Miura K, Narum DL, Ellis RD, Malkin E, Mullen GE, Miller LH, Martin LB, Pierce SK. The TLR9 ligand CpG promotes the acquisition of Plasmodium falciparumspecific memory B cells in malaria-naive individuals. J Immunol. 2009; 182:3318–26.
133. Traore B, Kone Y, Doumbo S, Doumtabe D, Traore A, Crompton PD, Mircetic M, Huang CY, Kayentao K, Dicko A, Sagara I, Ellis RD, Miura K, Guindo A, Miller LH, Doumbo OK, Pierce SK. The TLR9 agonist CpG fails to enhance the acquisition of Plasmodium falciparum-specific memory B cells in semi-immune adults in Mali. Vaccine. 2009; 27:7299–303.

134. Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghavi M, Lozano R, Lopez AD. Global malaria mortality between 1980 and 2010: a systematic analysis. Lancet. 2012; 379:413–31.
135. Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW. Malaria in pregnancy: pathogenesis and immunity. The Lancet infectious diseases. 2007; 7:105–17.
136. Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, Marsh V, Newton C, Winstanley P, Warn P, Peshu N, et al. Indicators of life-threatening malaria in African children. N Engl J Med. 1995; 332:1399–404.
137. Craig AG, Grau GE, Janse C, Kazura JW, Milner D, Barnwell JW, Turner G, Langhorne J. The role of animal models for research on severe malaria. PLoS Pathog. 2012; 8:e1002401.
138. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annu Rev Immunol. 2007; 25:697–743.
139. Marchiafava F, Bignami A. Sulle febbri malariche estivo-autunnali. Rome: Loescher; 1892.
140. Kyes S, Horrocks P, Newbold C. Antigenic variation at the infected red cell surface in malaria. Annu Rev Microbiol. 2001;55:673–707. doi: 10.1146/annurev.micro.55.1.673.
141. Scherf A, Lopez-Rubio JJ, Riviere L. Antigenic variation in Plasmodium falciparum. Annu Rev Microbiol. 2008;62:445–470. doi: 10.1146/annurev.micro.61.080706.093134.
142. Carvalho BO, Lopes SC, Nogueira PA, Orlandi PP, Bargieri DY, Blanco YC, Mamoni R, Leite JA, Rodrigues MM, Soares IS, Oliveira TR, Wunderlich G, Lacerda MV, del Portillo HA, Araújo MO, Russell B, Suwanarusk R, Snounou G, Rénia L, Costa. FT. On the cytoadhesion of Plasmodium vivax-infected erythrocytes. J Infect Dis. 2010;202:638–647. doi: 10.1086/654815.
143. Grau GE, Craig AG. Cerebral malaria pathogenesis: revisiting parasite and host contributions. Future Microbiol. 2012 Feb;7(2):291–302. doi: 10.2217/fmb.11.155.
144. Dondorp AM, Pongponratn E, White NJ. Reduced microcirculatory flow in severe falciparum malaria: pathophysiology and electron-microscopic pathology. Acta Trop. 2004;89:309–317. doi: 10.1016/j.actatropica.2003.10.004.
145. Chakravorty SJ, Craig A. The role of ICAM-1 in Plasmodium falciparum cytoadherence. Eur J Cell Biol. 2005;84:15–27. doi: 10.1016/j.ejcb.2004.09.002.
146. Turner GD, Morrison H, Jones M, Davis TM, Looareesuwan S, Buley ID, Gatter KC, Newbold CI, Pukritayakamee S, Nagachinta B. An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. Am J Pathol. 1994;145:1057–1069.
147. Milner DA., Jr Rethinking cerebral malaria pathology. Curr Opin Infect Dis. 2010;23:456–63. doi: 10.1097/QCO.0b013e32833c3dbe.

148. Fried M, Domingo GJ, Gowda CD, Mutabingwa TK, Duffy PE. Plasmodium falciparum: chondroitin sulfate A is the major receptor for adhesion of parasitized erythrocytes in the placenta. Exp Parasitol. 2006;113:36–42. doi: 10.1016/j.exppara.2005.12.003.
149. Reeder JC, Cowman AF, Davern KM, Beeson JG, Thompson JK, Rogerson SJ, Brown GV. The adhesion of Plasmodium falciparum-infected erythrocytes to chondroitin sulfate A is mediated by P. falciparum erythrocyte membrane protein 1. Proc Natl Acad Sci USA. 1999;96:5198–5202. doi: 10.1073/pnas.96.9.5198.
150. Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW. Malaria in pregnancy: pathogenesis and immunity. Lancet Infect Dis. 2007;7:105–117. doi: 10.1016/S1473-3099(07)70022-1.
151. von Itzstein M, Plebanski M, Cooke BM, Coppel RL. Hot, sweet and sticky: the glycobiology of Plasmodium falciparum. Trends Parasitol. 2008;24:210–218. doi: 10.1016/j.pt.2008.02.007.
152. Rowe JA, Claessens A, Corrigan RA, Arman M. Adhesion of Plasmodium falciparum-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. Expert Rev Mol Med. 2009;11:e16. doi: 10.1017/S1462399409001082.
153. Chen Q, Barragan A, Fernandez V, Sundström A, Schlichtherle M, Sahlén A, Carlson J, Datta S, Wahlgren M. Identification of Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) as the rosetting ligand of the malaria parasite P. falciparum. J Exp Med. 1998;187:15–23. doi: 10.1084/jem.187.1.15.
154. Vogt AM, Barragan A, Chen Q, Kironde F, Spillmann D, Wahlgren M. Heparan sulfate on endothelial cells mediates the binding of Plasmodium falciparum-infected erythrocytes via the DBL1alpha domain of PfEMP1. Blood. 2003;101:2405–2411. doi: 10.1182/blood-2002-07-2016.
155. Barragan A, Kremsner PG, Wahlgren M, Carlson J. Blood group A antigen is a coreceptor in Plasmodium falciparum rosetting. Infect Immun. 2000;68:2971–2975. doi: 10.1128/IAI.68.5.2971-2975.2000.
156. Rowe JA, Handel IG, Thera MA, Deans AM, Lyke KE, Koné A, Diallo DA, Raza A, Kai O, Marsh K, Plowe CV, Doumbo OK, Moulds JM. Blood group O protects against severe Plasmodium falciparum malaria through the mechanism of reduced rosetting. Proc Natl Acad Sci USA. 2007;104:17471–17476. doi: 10.1073/pnas.0705390104.
157. Udomsanpetch R, Thanikkul K, Pukrittayakamee S, White NJ. Rosette formation by Plasmodium vivax. Trans R Soc Trop Med Hyg. 1995;89:635–637. doi: 10.1016/0035-9203(95)90422-0.
158. Angus BJ, Thanikkul K, Silamut K, White NJ, Udomsangpetch R. Short report: Rosette formation in Plasmodium ovale infection. Am J Trop Med Hyg. 1996;55:560–561.

159. Doumbo OK, Thera MA, Koné AK, Raza A, Tempest LJ, Lyke KE, Plowe CV, Rowe JA. High levels of Plasmodium falciparum rosetting in all clinical forms of severe malaria in African children. Am J Trop Med Hyg. 2009;81:987–993. doi: 10.4269/ajtmh.2009.09-0406.
160. Le Scanf C, Vigan-Womas I, Contamin H, Guillotte M, Bischoff E, Mercereau-Puijalon O. Rosetting is associated with increased Plasmodium falciparum in vivo multiplication rate in the Saimiri sciureus monkey. Microbes Infect. 2008;10:447–451. doi: 10.1016/j.micinf.2007.12.012.
161. Uyoga S, Skorokhod OA, Opiyo M, Orori EN, Williams TN, Arese P, Schwarzer E. Transfer of 4-hydroxynonenal from parasitized to non-parasitized erythrocytes in rosettes Proposed role in severe malaria anemia. Br J Haematol. 2012.
162. Nussenblatt V, Semba RD. Micronutrient malnutrition and the pathogenesis of malarial anemia. Acta Trop. 2002;82:321–37. doi: 10.1016/S0001-706X(02)00049-9.
163. Ghosh K, Ghosh K. Pathogenesis of anemia in malaria: a concise review. Parasitol Res. 2007;101:1463–9. doi: 10.1007/s00436-007-0742-1.
164. Haldar K, Mohandas N. Malaria, erythrocytic infection and anemia. Hematology Am Soc Hematol Educ Program. 2009; 1: 87-93..
165. Skorokhod OA, Caione L, Marrocco T, Migliardi G, Barrera V, Arese P, Piacibello W, Schwarzer E. Inhibition of erythropoiesis in malaria anemia: role of hemozoin and hemozoin-generated 4hydroxynonenal. Blood. 2010,116:4328-37.
166. Perkins DJ, Were T, Davenport GC, Kempaiah P, Hittner JB, Ong'echa JM. Severe malarial anemia: innate immunity and pathogenesis. Int J Biol Sci. 2011, 7: 1427-42.
167. Pradhan P. Malarial anaemia and nitric oxide induced megaloblastic anaemia: a review on the causes of malarial anaemia. J Vector Borne Dis. 2009; 46: 100-8 PMid:19502689.
168. Nweneka CV, Doherty CP, Cox S, Prentice A. Iron delocalization in the pathogenesis of malarial anaemia. Trans R Soc Trop Med Hyg. 2010, 104: 17584.
169. de Mast Q, Syafruddin D, Keijmel S, Riekerink TO, Deky O, Asih PB, Swinkels DW, van ser Ven AJ. Increased serum hepcidin and alterations in blood iron parameters associated with asymptomatic P. falciparum and P. vivax malaria. Haematologica. 2010; 95: 1068-74.
170. Maina RN, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L, Jones D, Ogutu BR. Impact of Plasmodium falciparum infection on haematological parameters in children living in Western Kenya Malar J. 2010, 9: S4.
171. Kochar DK, Das A, Kochar A, Middha S, Acharya J, Tanwar GS, Gupta A, Pakalapati D, Garg S, Saxena V, Subudhi AK, Boopathi PA, Sirohi P, Kochar SK. Thrombocytopenia in Plasmodium falciparum, Plasmodium vivax and mixed infection malaria: a study from Bikaner (Northwestern India). Platelets. 2010, 21: 6237.

172. Leowattana W, Tangpukdee N, Thar SK, Nakasiri S, Srivilarit S, Kano S, Wilairatana P, Krudsood S. Changes in platelet count in uncomplicated and severe falciparum malaria. Southeast Asian J Trop Med Public Health. 2010, 41: 1035-41 PMid:21073022 57.
173. Tan SO, McGready R, Zwang J, Pimanpanarak M, Sriprawat K, Thwai KL, Moo Y, Ashley EA, Edwards B, Singhasivanon P, White NJ, Nosten F. Thrombocytopaenia in pregnant women with malaria on the Thai-Burmese border. Malar J. 2008, 7:209.
174. Lacerda MV, Mourão MP, Coelho HC, Santos JB. Thrombocytopenia in malaria: who cares? Mem Inst Oswaldo Cruz. 2011, 106: 52-63.
175. de Mast Q, Groot E, Asih PB, Syafruddin D, Oosting M, Sebastian S, Ferwerda B, Netea MG, de Groot PG, van der Ven AJ, Fijnheer R. ADAMTS13 deficiency with elevated levels of ultra-large and active von Willebrand factor in P. falciparum and P. vivax malaria. Am J Trop Med Hyg. 2009; 80: 492-8 PMid:19270304.
176. de Mast Q, de Groot PG, van Heerde WL, Roestenberg M, van Velzen JF, Verbruggen B, Roest M, McCall M, Nieman AE, Westendorp J, Syafruddin D, Fijnheer R, van Dongen-Lases EC, Sauerwein RW, van der Ven AJ. Thrombocytopenia in early malaria is associated with GP1b shedding in absence of systemic platelet activation and consumptive coagulopathy. Br J Haematol. 2010; 151: 495-503.
177. Sahu S, Mohanty NK, Rath J, Patnaik SB. Spectrum of malaria complications in an intensive care unit. Singapore Med J. 2010; 51: 226-9 PMid:20428736.
178. Saleri N, Gulletta M, Matteelli A, Caligaris S, Tomasoni LR, Antonini B, Perandin F, Castelli F. Acute Respiratory Distress Syndrome in Plasmodium vivax malaria from Venezuela. Journal of Travel Medicine, 2006; 13: 112-113.
179. Sharma A, Khanduri U. How benign is benign tertian malaria? J Vector Born Dis. 2009, 46: 141-4 PMid:19502694.
180. Anand AC, Ramji C, Narula AS, Singh W. Malarial hepatitis: a heterogeneous syndrome? Natl Med J India. 1992, 5: 59-62 PMid:1304265.
181. Mazumder R, Mishra RK, Mazumder H, Mukherjee P. Jaundice in falciparum malaria - some prospective observations. J Indian Med Assoc. 2002, 100: 312-4 PMid:12418632.
182. Murthy GL, Sahay RK, Sreenivas DV, Sundaram C, Shantaram V. Hepatitis in falciparum malaria. Trop Gastroenterol. 1998, 19: 1524 PMid:10228440.
183. Sung YH, Park JM. A case of malarial hepatitis by Plasmodium vivax. Korean J Gastroenterol. 2010; 56: 329-33.
184. Das SN, Mohapatra B, Mohanty R, Dash PC, Kar K, Dash PK. Malarial hepatitis as a component of multiorgan failure – a bad prognostic sign. J Indian Med Assoc. 2007; 105: 247-50 PMid:17915792.
185. Ali H, Ahsan T, Mahmood T, Bakht SF, Faroog MU, Ahmed N. Parasite density and the spectrum of clinical illness in falciparum malaria. J Coll Physicians Surg Pak. 2008; 18: 362-8 PMid:18760048.

186. Anand AC, Puri P. Jaundice in malaria. J Gastroenterol Hepatol. 2005, 20: 1322-32.
187. Kochar DK, Singh P, Agarwal P, Kochar SK, Pokharna R, Sareen PK. Malarial hepatitis. J Assoc Physicians India 2003, 51: 1069-72 PMid:15260391.
188. Naicker S, Aboud O, Gharbi MB. Epidemiology of acute kidney injury in Africa. Semin Nephrol. 2008, 28: 348-53.
189. Lombardi R, Yu L, Younes-Ibrahim M, Schor N, Burdmann EA. Epidemiology of acute kidney injury in Latin America. Semin Nephrol. 2008; 28: 320-9.
190. Jha V, Chungh KS. Community-acquired acute kidney injury in Asia. Semin Nephrol. 2008; 28: 330-47.
191. Assounga AG, Assambo-Kieku C, Mafoua A, Moyen G, Nzingoula S. Etiology and outcome of acute renal failure in children in Congo-Brazzaville. Saudi J Kidney Dis Transplant. 2000; 11: 40-3 PMid:18209297.
192. Ehrich JHH, Eke FU. Malaria-induced renal damage: facts and myths. Pediatr Nephol. 2007; 22: 626-37.
193. Barsoun RS. Malaria nephropathies. Nephrol Dial Transplant. 1998;, 13: 1588-97.
194. Khan FY. An imported case of P. falciparum malaria presenting as black water fever with acute renal failure. Travel Med Infect Dis. 2009; 7: 378-80.
195. Oumar AA, Poudiougou B, Sylla M, Sall A, Konate S, Togo B, Diakite M, Keita MM. Black water fever in children during cerebral malaria: 3 case-reports in Bamako. Arch Pediatr. 2007, 14: 993-5.
196. Rogier C, Imbert P, Tall A, Sokhna C, Spiegel A, Trape JF. Epidemiological and clinic aspects of black water fever among African children suffering frequent malaria attacks. Trans R Soc Trop Med Hyg. 2003, 97: 193-7.
197. Bardaji A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, Mabunda S, Alonso PL, Menendez C. Impact of malaria at the end of pregnancy on infant mortality and morbidity. J Infect Dis. 2011, 203: 691-9.
198. Uneke CJ. Impact of Placental Plasmodium falciparum malaria on pregnancy and perinatal outcome in Sub-Saharan Africa. Part III: placental malaria, maternal health and public health. Yale J Biol Med. 2008; 81: 1-7 PMid:18604306 PMCid:2442721.
199. Aribodor DN, Nwaorgu OC, Eneanya CI, Okoli I, PukkilaWorley R, Etaga HO. Association of low birth weight and placental malarial infection in Nigeria. J Infect Dev Ctries. 2009; 3:620-3.
200. Adam I, Elhassan EM, Haggaz AE, Ali AA, Adam GK. A perspective of the epidemiology of malaria and anaemia and their impact on maternal and perinatal outcomes in Sudan. J Infect Dev Ctries. 2011, 5:083-7.
201. Carvalho BO, Matsuda JS, Luz SL, Martinez-Espinosa FE, Leite JA, Franzin F, Orlandi PP, Gregoracci GG, Lacerda MV, Nogueira PA, Costa FT. Gestational malaria associated to Plasmodium vivax and Plasmodium falciparum placental mixed-infection

followed by foetal loss: a case report from an unstable transmission area in Brazil. Malar J. 2011; 10: 178.
202. Uneke CJ. Impact of Placental Plasmodium falciparum malaria on pregnancy and perinatal outcome in Sub-Saharan Africa. I: Introduction to placental malaria. Yale J Biol Med. 2007; 80: 39-50 PMid: 18160989 PMCid:2140183.
203. Bell D , Wongsrichanalai C , Barnwell JW . Ensuring quality and access for malaria diagnosis: how can it be achieved? *Nature Rev Microbiol*. 2006;4:682–695.
204. WHO Global Malaria Programme. *World Malaria Report 2010*. Geneva, Switzerland: World Health Organization; 2011.
205. Murray CK , Gasser RA , Magill AJ , Miller RS . Update on rapid diagnostic testing for malaria. *Clin Microbiol Rev*. 2008;21:97–110.
206. Gillet P , Maltha J , Hemans V , Ravinetto R , Bruggeman C , Jacobs J . Malaria rapid diagnostic kits: quality of packaging, design and labelling of boxes and components and readability and accuracy of information inserts. *Malar J*. 2011;10:39
207. *Malaria Rapid Diagnostic Test Performance: Results of WHO Product Testing of Malaria RDTs: Round 3 (2010–2011)*. Geneva, Switzerland: World Health Organization: 2011.
208. Lubell Y , Reyburn H , Mbakilwa H , et al. The impact of response to the results of diagnostic tests for malaria: cost-benefit analysis. *BMJ*. 2008;336:202–205.
209. Ocholoa LB , Smith T , Mabaso MLH , Newton CRJC . The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard. *Lancet Infect Dis*. 2006;6:582–588.
210. Nakamura K-I , Rabbege JR , Baird JK , et al. Malaria. In: Connor DH , Chandler FW , Manz HJ , Schwartz DA , Lack EE , eds. *Pathology of Infectious Diseases*. Stamford, CT: Appleton & Lange;1997:1205–1221.
211. Polage CR , Bedu-Addo G , Owusu-Ofori A , et al. Laboratory use in Ghana: physician perception and practice. *Am J Trop Med Hyg*. 2006;75:526–531.
212. McMurrow ML , Aidoo M , Kachur SP . Malaria rapid diagnostic tests in elimination strategies—can they find the last parasite? *Clin Microbiol Infect Dis*. 2011;17:1624–1631.
213. https://www.cdc.gov/malaria/resources/pdf/clinicalguidance.pdf
214. https://www.niaid.nih.gov/diseases-conditions/malaria-strategies.
215. Doolan, D.L., Dobaño, C., Baird, J.K. *Clin Microbiol Rev*. 2009 Jan;22(1):13-36, Table of Contents. Acquired immunity to malaria.
216. Nussenzweig, R.S., Vanderberg, J., Most, H., Orton, C. (1967). Protective immunity produced by the injection of x-irradiated sporozoites of Plasmodium berghei. *Nature.* 216:160 – 162.
217. Hoffman et al. Proctection of humans against maleria by immunization with radiation-atteuated Plasmodium falciparum sporozoites. *J Inf Dis* 185:1155–1164.
218. MVI Website.

219. MVI. Vaccine development. Accessed 01/
220. HOV. Vaccine development, testing, and regulation.
221. http://www.malariavaccine.org/malvac-approaches.php.
222. http://www.malariavaccine.org/malvac-approaches.php