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Malaria

Arbeitskreis Blut, Untergruppe «Bewertung Blutassoziierter Krankheitserreger»

1 Current Knowledge about the Pathogen

Malaria occurs world-wide in more than 100 countries in the tropical and subtropical regions and is the most significant parasitic disease in humans. Each year, it causes disease in approximately 500 million people and kills between 1 and 3 million people, above all children under 5 years. Four different types of protozoae are responsible for the disease. They belong to the genus *Plasmodium* (class haematozoea, order haemosporida). These include:

– *Plasmodium falciparum* – the agent of tropical malaria
– *Plasmodium vivax* und *Plasmodium ovale* – the agent of tertian malaria.
– *Plasmodium malariae* – the agent of quartan malaria.

P. falciparum consists of genetically different types and, based on the analyses of core and mitochondria nucleic acid sequences, is the agent most closely related to *Plasmodium gallinaceum.* It probably originates from Africa [1]. As can be derived from the mutations found, the age of *P. falciparum* is estimated to be 10,000–100,000 years which corresponds to the evolution of the hominids since in Central and North Europe no markers can be found for selection pressure by malaria [2]. Humans are the only hosts for these four human pathogenic *Plasmodium* species. In rare cases, infections are caused by malaria parasites of other primates (e.g. *Plasmodium knowlesi, Plasmodium cynomolgie* and *Plasmodium simium*).

1.1 Characteristics of Malaria

Plasmodium is a parasite that grows intracellularly. The life cycle of plasmodium develops in two phases: an asexual phase in the human host and a sexual phase in the carrier, the *Anopheles* mosquito. The sporozoites transmitted during a blood meal rapidly penetrate from the blood stream into the liver parenchymal cells in which they replicate asexually. Depending on the plasmodium species, this so-called schizogony phase lasts between 5–7 days in *P. falciparum* and between 6–18 days in the other species. Schizogony, formerly referred to as merogony, is the asexual replication of the protozoae. A schizont contains several cell nuclei; the daughter nuclei are surrounded by cytoplasm and organise themselves into single individuals, the merozoites. One single sporozoite can produce between 10,000 and more than 30,000 merozoites. For *P. vivax* and *P. ovale*, part of the schizonts remain in a kind of inactive phase (hypnozoites); they can remain in the liver cell for months or years, and may then lead to the relapses characteristic of tertian malaria.

After schizogony is completed, the swollen liver cell ruptures and releases the mobile merozoites into the blood stream. These adhere to the red blood cells via specific surface receptors (receptor in the case of *P. vivax* e.g. Duffy blood group antigen, Fy^a or Fy^b, or in case of *P. falciparum* glycophorin A). Then, they enter the red blood cells and turn into trophozoites. At the end of the 48- to 72-hour erythrocytic phase, the schizonts will have formed in the red blood cells. During this phase, so-called seal-ring shapes (vacuoles with parietal nuclei) may form (cf. fig. 1). From decayed red blood cells, new merozoites may be released which can infect further red blood cells. A part of the merozoites differentiates within erythrocytes into sexual stages, forming macro- and microgametocytes. In the intra-erythrocytic vacuoles, haemozoin is formed as an insoluble metabolite of haemoglobin, called malaria pigment.

After ingestion of male and female gametocytes during a blood meal, a motile flagellated zygote is formed in the midgut of the *Anopheles* mosquito. This zygote moves into the salivary gland. An oocyst is formed releasing sporozoites which can infect a new human host via the saliva of the mosquito.

1.2 Infection and Infectious Disease [3–5]

Malaria infection in humans is caused by a sting of the female *Anopheles* mosquito during which sporozoites are released

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from the salivary gland of the mosquito into the organism of the host during a blood meal. The sporozoites then go through the cycle described in 1.1 'Characteristics of Malaria'. The symptoms in humans are caused by the invasion and destruction of the red blood cells by the asexual parasites and the immune response of the host. The red blood cells are affected by strong consumption and degradation of intracellular proteins, especially of haemoglobin, caused by the growing parasites. Changes occur in the membrane of the red blood cell, and their deformability is reduced. Above all, the adhesion protein PfEMP-1 (*P. falciparum* erythrocyte membrane protein-1) plays an important part in the pathogenesis of *P. falciparum*. This protein mediates the adherence to the receptors of the venous and capillary endothelium (cell adherence). Around 60 different var-genes encode for different variants of PfEMP-1, each with individual antigenic and adhesive properties.

It is assumed that one particular PfEMP-1 each is prevalent on the surface of one individual infected red blood cell. Receptor molecules for PfEMP-1 above all include the intercellular adhesion molecule 1 (ICAM-1) in the brain, chondroitin sulphate B in the placenta, and CD36 in other organs. Infected red blood cell can also adhere to other non-affected red blood cells (rosette formation) and other infected red blood cells (agglutination). Cell adhesion and rosette formation will lead to sequestration of the red blood cells which contain mature forms of the parasite in the capillaries of different organs (especially the brain). The impairment of microcirculation, caused by the cell adherence of the red blood cells amongst each other and the cell adherence to the endothelium, the resulting reduction of blood flow in the capillaries, the possible intravasal coagulation activation and the changes in the metabolism will lead to reduced oxygen supply in the brain, kidneys, liver and lungs, which are responsible for the severe course and deadly complications of tropical malaria.

The symptoms of acute malaria are non-specific and begin no sooner than 6 days after a sting by an infected mosquito during the intraerythocytic phase of the development cycle. The symptoms which first occur include malaise, headache, abdominal pain, limb and muscle pain as well as fever. Frequent symptoms are nausea, vomiting, diarrhoea, and orthostatic hypotension. In case of tropical malaria caused by *P. falciparum*, the fever occurs irregularly while symptomatic phases with chills and fever in 48- or 72-hour cycles occur in case of infections with *P. vivax, P. ovale* or *P. malariae* (tertian or quartan malaria). Individuals who are not immune may develop body temperatures of 40 °C and higher. In many cases, thrombopenia is present, and in addition, splenomegaly and hepatomegaly as well as diarrhoea in 30% of the cases.

If tropical malaria is left untreated or is inappropriately treated, complicated malaria can develop in different organs due to sequestration of infected red blood cells. Characteristic phenomena for such complicated courses of tropical malaria include impairments of consciousness up to coma, often in connection with seizures (cerebral malaria), hypoglycaemia, lactic acidosis, impairment of the kidney and liver functions, ARDS (adult respiratory distress syndrome) and haematological changes, especially pronounced haemolytic anaemia. In addition, coagulation disorders (only very seldom as disseminated intravasal coagulation), thrombocytopenia and haemoglobinuria may occur. The pathogenetic mechanisms underlying the symptoms of tropical malaria are only partly known and include disorders of the microcirculation (cerebral malaria, impairment of the kidney function), anaerobic glycolysis (lactic acidosis), haemolysis (anaemia) and disorders of the metabolism such as reduced gluconeogenesis in the liver, hyperinsulinaemia and increased glucose consumption by parasites (hypoglycaemia).

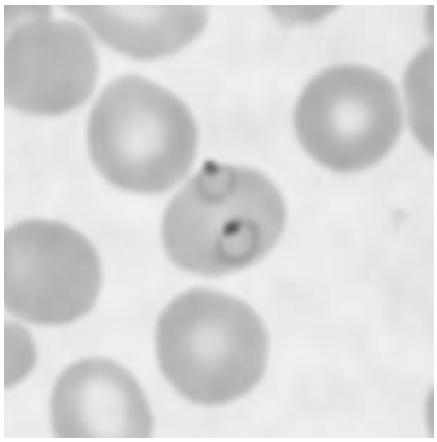

Fig. 1. *Plasmodium falciparum* ring shapes in thin blood smear.

The most severe course of tropical malaria is cerebral malaria. This is the course which is in most cases responsible for the high lethality, despite appropriate treatment, and for neurological deficits, especially in children. During pregnancy, tropical malaria is related to increased maternal and foetal morbidity and lethality. In semi-immune primigravid and secundigravid women, cerebral malaria results in low birth weight and increased infant and child mortality due to reduced oxygen supply. An HIV infection of the mother predisposes for increased parasitaemia and increases the risk of connatal malaria. In non-immune pregnant women, the risk of infection with severe outcome and high parasitaemia and anaemia is high. Severe tropical malaria, as a rule, leads to a miscarriage. Connatal malaria occurs in less than 5% of the newborns of infected mothers in endemic areas.

The clinical course of tropical malaria in children resembles that of adults. Above all in infants more than 6 months of age, who no longer have a certain protection from malaria by maternal antibodies, and in small children, the course is often serious with a high complication rate in contrast to adults, with serious anaemia causing cerebral malaria. According to WHO estimates, more than half the estimated 1–3 million annual deaths in African children is caused by tropical malaria.

Clinical symptoms of tertian malaria caused by *P. vivax* resembles tertian malaria caused by *P. vivax*. Both parasites infect only young red blood cells (reticulocytes) so that parasitaemia

Table 1. Time from end of travel up to the occurrence of symptoms in cases of malaria reported in accordance with the IfSG (Infection Protection Act) who did not stay in the country of travel for longer than 8 weeks

Form of malaria	Number of cases from 2001–2006	Number of cases with information on end of travel and beginning of symptoms	Months after end of travel	Portion of cases with symptoms, %
Tropical	3,334	1,446	2	98.8
			6	99.8
			12	99.9
			13	100
Tertian	796	261	2	55.6
			6	85.4
			12	97.7
			60	100
Quartan	103	33	2	81.8
			6	100

in the blood is detectable in only 1–2% of the red blood cells. Symptoms that may occur include anaemia due to haemolysis, thrombocytopenia and in rare cases rupture of the spleen. Microcirculatory disorders due to sequestration of the red blood cells may also occur very seldom.

Because of the occurrence of dormant parasite forms in the liver (hypnozoites), relapses can occur after remitting tertian malaria after up to 4 years. In extreme cases, even longer relapse periods were observed.

Quartan malaria which is caused by *P. malariae* as a rule has a benign course with only mild symptoms. The parasites infect predominantly old red blood cells; the result is low parasitaemia (1–2%) in the blood, as in tertian malaria. Glomerulonephritis due to chronic formation of immune complexes with deposits in the kidney may occur. Unlike tertian malaria, quartan malaria displays no hypnozoites in the liver and therefore no latent hepatic courses. Late quartan malaria attacks (recrudescences) may result from persisting forms of *P. malariae* in blood vessel endothelia [5]. Quartan malaria can persist for up to 40 years.

Chronic complications of malaria include the tropical splenomegaly syndrome, the nephrotic syndrome in case of quartan malaria and possibly an increased occurrence of Epstein-Barr virus (EBV) associated Burkitt lymphoma during childhood.

1.3 Epidemiology

According to the WHO, there were possible transmissions of malaria in a total of 107 countries in 2004 [6]. Although this figure has declined since the 1950s (140 countries with endemic malaria), roughly 3.2 billion people currently live at a permanent risk of malaria. It is estimated that every year between 350 and 600 million clinical cases of malaria occur world-wide, out of which 60% occur in Africa south of the Sahara alone. Out of the more than 1 million deaths in Africa caused by malaria, roughly half are children under 5 years.

Almost all of these deaths are due to tropical malaria. *P. falciparum* mainly occurs in Africa and in certain regions of South-East Asia, the Caribbean and South America. The second most frequent malaria species, *P. vivax*, is found in wider parts of Asia, America, and North Africa. Altogether, more than 40 different *Anopheles* mosquito species are able to transmit malaria. The most important malaria vector, *Anopheles gambiae*, occurs exclusively in Africa.

The extent of endemic occurrence of malaria is calculated from the degree of parasitaemia or the rate in children between 2 and 9 years with an enlargement of the spleen. If the rate is below 10%, we talk about hypoendemic, between 11 and 50% about mesoendemic, between 51 and 75% of hyperendemic and with a portion of spleen enlargements of over 75% of holoendemic regions. In certain holo- and hyperendemic regions of tropical Africa or New Guinea with a very high transmission rate of *P. falciparum*, the inhabitants are repeatedly infected with plasmodia throughout their lifetime. In these regions, there is a high morbidity and mortality during childhood. Whereas health impairments are serious in children, malaria infections in adults frequently show few symptoms because of the adult's partial immunity. Such a situation with frequent re-infections throughout the entire year is described as 'stable malaria' and occurs in holo- and hyperendemic regions. In hypoendemic regions, the transmission rate is low, and infections only occasionally occur seasonally or in certain districts. Depending on external circumstances (e.g. rainy season), the incidence of malaria can be markedly increased, and epidemics can occur. In general, the epidemiology of malaria is influenced by the number of infectious stings, the mosquito density, the number of infected mosquitoes, the number of chronically infected individuals, the degree of anthropophilia of the vector, the interval between the blood meals, the lifetime of the mosquitoes and the sporozoite infection dose. The ambient temperature also plays an important role, since development of human pathogenic plasmodia persists below 16 °C. Thus, an endemic spread of *P. vivax* as occurred about 100 years ago in Central Europe is no longer

possible since the human host is missing thanks to a successful treatment of malaria.

In Germany, altogether 628 cases of malaria were reported in 2005 pursuant to the § 7 IfSG (Infektionsschutzgesetz; German Infection Protection Act). This corresponds to an incidence of 0.8 cases of infection per 100,000 inhabitants. In the years before 2005, the reported figures were 707 cases (2004), 820 cases (2003), 859 cases (2002), 1,045 cases (2001), 836 cases (2000), 931 cases (1999), and 1,008 cases (1998) [7]. The largest portion of malaria infections in 2005 was imported from African countries (88%). 7% of the reported cases originated from Asia, 3% from America, and 2% from Australia/Oceania. Among those countries from which malaria was imported, Ghana, Nigeria, Cameroon and Kenya were at the top of the list. In 78% of the malaria cases reported in 2005, *P. falciparum* was identified as the agent of tropical malaria while *P. vivax* ranked second with 12%, and *P. ovale* and *P. malariae* were registered with only 4 and 3%. Among the total of 6 deaths by tropical malaria in 2005, three cases had infection with *P. falciparum*, one case had a mixed infection, and in 2 cases no pathogen species was identified. One of the individuals originated from Cameroon, and 5 were German. Evidence showed that 2 of the individuals who died had not performed chemoprophylaxis. The countries of origin indicated were Cameroon, Gambia, Senegal and Ghana.

Out of the cases of malaria reported to the Robert Koch Institute for 2001 to 2006 (as of 12 July 2007), altogether 4,639 cases fulfilled the reference definition. For part of the cases reported, both the time of travel and the onset of symptoms of the disease were available. Although no exact incubation period can be derived from these data, the time of the onset of clinical symptoms after returning from an endemic area is important for blood safety concerns, i.e. the determination of deferral periods. This especially applies to infection with *P. vivax* and *P. ovale* as well as *P. malariae*, since these may have longer incubation periods. An analysis of the data on individuals who did not stay in an endemic area for malaria for more than 8 weeks (individuals who do not have their temporary centre of activity there), shows that in 14.6% of the returnees reported on tertian malaria in accordance with the IfSG symptoms occurred only after more than 6 months (table 1). Similar information was also obtained from Switzerland [8].

The rare cases of malaria infections acquired in non-endemic areas are either transfusion associated malaria cases (cf. 3 'Recipients') or so-called airport malaria and/or nosocomial transmissions. Airport malaria is transmitted during the flight or a stop-over and/or by mosquitoes e.g. transported in the luggage [9]. Nosocomial malaria transmissions can be caused e.g. by needle injuries, contaminated fluids and contaminated medical devices [10–13].

Besides, autochthonous malaria transmissions (i.e. transmissions which developed on site) can occur in regions in which malaria does not usually exist or has been eradicated if the respective vector is present (*Anopheles* spp.). Thus, 7 locally restricted transmissions of *P. vivax* were reported in Palm State County, FL, USA, in 2003 [14].

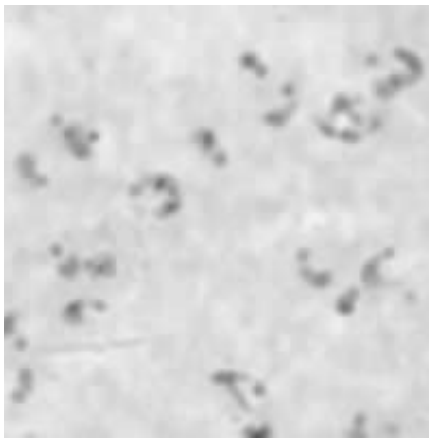

Fig. 2. *Plasmodium falciparum* ring shapes in thick drop.

Anopheles spp. native to Germany, too, can transmit plasmodia; depending on ambient temperature, infectious chains are therefore well possible in Germany and have occurred before. *P. vivax* and tertian malaria were wide-spread in Southern Germany up to the middle of the 19th century. At the Upper Rhine, malaria did not recede before the straightening of the Rhine which reduced the breeding grounds for *Anopheles*. Sporogeny of *P. vivax* in *Anopheles* occurs up to a summer isotherm of 16 °C. The main areas of distribution are within the 25 °C summer isotherm which runs through the middle of Germany [15]. In the past few decades, no case of endemic malaria has been reported in Germany. The last autochthonic cases of malaria were observed up to around 1950 in Berlin and its surroundings [16].

Malaria caused by *P. falciparum* has recently been reported in 2 German children, who had no history of international travels but stayed at a hospital at the same time as a child from Angola infected with tropical malaria. Since breeding grounds of *Anopheles plumbeus* (a potential plasmodium carrier) were found in the vicinity of the hospital and, in addition, temperatures were between 21 and 27 °C on average during the day time, transmission by *A. plumbeus* was assumed as the possible cause [17].

1.4 Detection Methods and Their Significance

1.4.1 Thick Drop and Blood Smear

The gold standard for malaria diagnostics is still the microscopic examination of the so-called thick drop or thin blood smears stained in accordance with May-Grünwald or Wright-Giemsa, respectively [18], or by means of fluorescence colouring (figs 1, 2). In the thick drop method, plasmodia are enriched to the 6- to 10-fold concentration compared with the blood smear. The degree of parasitaemia/µl can be determined

by means of the parasite and leucocyte count via a correlation with the total leucocyte count. A negative test result does not reliably exclude a malaria infection since at the beginning of clinical manifestation the parasite density might be low in the peripheral blood. In case of continued clinical suspicion and negative results, the test must be repeated several times, e.g. at 12-hour intervals. The experience of the investigator plays an important part in malaria diagnostics. The detection limit is 5–10 parasites/µl. In the case of less experienced investigators, it is increased to the power of 10.

1.4.2 NAT Method

Various investigators have compared in particular the sensitivity of NAT tests with conventional microscopy after staining in accordance with Giemsa or Wright-Giemsa. All studies showed a higher sensitivity after DNA extraction by means of NAT. Around 5–10 µl blood are used for blood smear or thick drop; for DNA extraction, the quantity is 200 µl.

For the conventional nested PCR, different primers are used to include all plasmodia species. These primers bind in the 18s RNA [19, 20]. Sensitivity of microscopy is approximately 93% if 100% are set in the PCR. Other methods included real-time PCR [21] and real-time PCR with probes as molecular beacon [22]. An arithmetic sensitivity of 0.004 parasites/µl blood was obtained for *P. falciparum*, and for genus-specific PCR this figure was 0.16/µl [22]. Compared with microscopy, a sensitivity of 97.4% was obtained with genus-specific sensitivity [23]. According to another report, no cross-reaction with *Toxoplasma gondii* and *Leishmania infantum* was found at a sensitivity of 0.7 parasites/µl for *P. falciparum*, 4 parasites/µl for *P. vivax*, and 1.5 parasites/µl for *P. ovale* [24].

1.4.3 Plasmodium Antigen Detection

The first few tests for the determination of plasmodium components showed low sensitivity and specificity [25–27], even though parasitic LDH was used as antigen. Rapid tests were developed to support travellers in malaria diagnostics in the event of fever or exposure. Direct detection tests for plasmodium have been improved in the meantime so that a sensitivity of 85% and a specificity of 96% can be reached for *P. falciparum* infections [28]. Low parasite density could be a reason for low sensitivity. The antibody cross-reacts with *P. vivax*, but at a low sensitivity. When antibodies were used against histidine-rich protein II of *P. falciparum*, a sensitivity of 97% and a specificity of 96% were obtained in travel returnees [29].

Depending on the exposure, a sensitivity of 88% and a specificity of 99% can be reached for *P. falciparum*. For *P. vivax*, the respective figures are 76% and 100%[30]. Monoclonal antibody based ELISA has shown a sensitivity of 90% for *P. falciparum* in Thailand and Nepal [31].

The quality of the plasmodium indicated in each study quoted depends on the comparative tests used in these studies, i.e. PCR or microscopy, and on the investigator's expertise in interpreting the test result.

1.4.4 Diagnostics Using Anti-Plasmodium Antibodies

Most of the studies published in this field deal with the determination of antibodies which are formed after a vaccination or which can be used for the appropriate antigens for a putative vaccine. The cytoadherence-linked asexual gene 9 (clag 9) [32], merozoite surface proteins 6 and 7 (MSP6, MSP7) [33] and the variant surface antigens (VSA) are immunogenic and can still be detected 10 years after infection [34].

Exposure to malaria parasites which was measured via IgM and IgG responses using a test containing 5 different proeryrthocyte antigens was published by some working groups from France in 2006. The test antigens were circumsporozoite protein, sporozoite threonine- and asparagine-rich protein, sporozoite and liver stage antigen, liver stage antigen 1 and SR11.1. The immune response was measured in 106 individuals 3 months after exposure and seemed plausible [35]. If leucin-rich protein is used, cross-reactions occur between *Schistosoma mansoni* and *P. falciparum* [36].

Since the infection with *P. falciparum* in the blood causes serious clinical symptoms, the testing of returnees from tropical regions after a delay of several months in the case of an asymptomatic state of health is immaterial from an epidemiological point of view. Testing for anti-plasmodium antibodies has more informational value as regards the exposure and possible circulation if no clinical abnormality has shown.

2 Blood and Plasma Donors

2.1 Prevalence and Incidence in Donor Populations

Little is known about the prevalence and incidence of malaria in blood donors in Germany. In an up-to-date study by Okocha et al. [37], a prevalence of antibodies of 30.2% was determined in donors in Nigeria. In Venezuela, 890 blood donors were studied by Nunez et al. [38] by means of ELISA. The total antibody prevalence found was 1.7%.

2.2 Definition of Exclusion Criteria

Conforming to the guidelines of the Bundesärztekammer (German Medical Association) on haemotherapy, individuals in Germany are excluded from donating blood for a period of 4 years following medically documented recovery from malaria [39]. In addition, persons who were born or raised in a malaria endemic area or temporarily had their centre of life in such an area are deferred from donating blood for a total of 4 years after their last stay in the endemic area. Before donating blood, infectiveness has to be excluded by means of a validated immunological or nucleic acid test. Persons visiting a malaria endemic area for a short period are not allowed to donate blood for a period of at least 6 months following their stay. The decision of a possible deferral is independent from

the occurrence of febrile episodes. For persons exclusively donating plasma for fractionation, exclusion from the donation due to a malaria risk need not be considered.
In the UK, potential donors who had a malaria infection or fever of unclear origin within 6 months after returning from an endemic area, or after having stayed in a malaria endemic area during the past 12 months or for an uninterrupted period of more than 6 months, are excluded from the donation. A negative test result for anti-plasmodium antibodies is required for re-admittance to the donor panel 3 years after the end of treatment or after 6 months free of symptoms after returning from the endemic area [40].
In France, antibody tests (indirect immunofluorescence antibody test; IFAT) are also performed within a period of between 4 months and up to 3 years after returning from an endemic area. The test algorithm depends on the duration of the stay in the malaria endemic area.

2.3 Donor Testing and Significance

Because of existing exclusion rules, no donor screening for plasmodium is carried out in Germany.

2.4 Donor Interviews

In countries with endemic occurrence of malaria in which a high portion of the donors is infected, the kind of donor exclusion which is common practice in non-endemic countries cannot be performed because a lack of donated blood supply would result. Besides, a great number of recipients have partial immunity. In some malaria endemic countries, recipients received administrations of chloroquine or, in the event of chloroquine resistance, sulfadoxin-pyrimethmin in order to prevent transfusion associated malaria. However, this type of preventative measure is not reliable in the very regions of Central and West Africa as well as South-East Asia due to increased resistance against these two substances.
When potential donors in Germany are interviewed, they are asked whether they originated or grew up in a malaria endemic region or whether they stayed in such a region in the past 6 months. A possible malaria prophylaxis is not taken into account here. In addition, the donor is obliged to indicate whether he/she has an acute malaria infection or ever had a malaria infection.

2.5 Donor Information and Counselling

Information or advice for a donor concerning malaria is not provided. If the donor is suspected to be infected with malaria, further clarification by a physician experienced in tropical medicine is required.

3 Recipients

3.1 Prevalence and Incidence of Blood-Associated Infections and Infectious Diseases in Recipient Populations

The first transmission of malaria by blood transfusion was described in 1911 in a patient with pernicious anaemia [41]. Although the donor indicated that he had never suffered from malaria, the recipient developed a febrile reaction 11 days following the donation, and *P. vivax* could be detected both in the donor and in the recipient. More than roughly 350 malaria infections following blood transfusions were reported for the years between 1910 and 1950 [42]. An analysis of the cases that became known between 1911 and 1979 [43] showed an increase in the incidence of malaria from 6 to 145 cases per year, parallel to the increasing number of transfusions. At first, *P. vivax* was the most frequently observed species in case of transfusion associated malaria while *P. malariae* prevailed in the 1950s. In the 1970s, *P. vivax* was again the prevalent species in cases of transfusion associated malaria followed by *P. malariae* and *P. falciparum*. Since the 1980s, however, *P. falciparum* has been the most frequently registered species of malaria following blood transfusions in countries in which malaria does not occur endemically (e.g. USA, Canada and UK). Thus, the portion of *P. falciparum* in the UK has risen from 37% in 1984 to 55% in 1993 [44].
Altogether 93 cases of transfusion associated malaria have been documented in the USA during the period from 1963 to 1999 [45]. 35% could be ascribed to *P. falciparum*, 27% to *P. vivax*, 27% to *P. malariae*, 5% to *P. ovale* and 3% to mixed infections. 11% of the patients died. Of 91 identified donors altogether, 67 could be associated with the transmission. 59% of the donors whose country of origin was known came from endemic areas. The estimated incidence of transfusion associated malaria in the USA is one case per 1 million donors. Around 1–3 cases per year are reported in the US Center for Disease Control and Prevention (CDC). It is assumed that the frequency of transfusion associated malaria in malaria endemic countries is more than 50 cases per 1 million donors. In Canada, the frequency of transfusion associated malaria is estimated to be 1 case per 4 million transfused red blood cell concentrates [46]. The three cases of tropical malaria published in 2001 were all transmitted by donors originating from malaria endemic areas but had lived in Canada symptom free for several years. Based on these 3 cases, the Canadian donor deferral criteria were amended to exclude persons who had recovered from malaria (2 of the 3 cases) permanently from donation.
Up to 1965, a total of 12 cases of transfusion associated malaria were reported from Germany [47–53]. The cases in question were 2 infections with *P. falciparum* and 7 infections with *P. vivax*, the 3 remaining infections could not be reliable assigned [overview in 42].
In the past few years, 3 reports were published on transfusion associated cases of malaria [54–56]. In 1998, Witt et al. [56] re-

ported on an 18-month-old boy who developed antibiotic refractory fever 14 days after a heart operation. On day 23, postoperative, intraerythrocytic ring shapes and gametocytes of *P. falciparum* were found in the peripheral blood smear. The child recovered rapidly after a 3-day administration of quinine and halofantrine. The retrospective analysis of the deferred samples of 7 donors from which the child had received red blood cell concentrates showed antibodies against *P. falciparum* in one of the donors.

Malaria caused by *P. falciparum* with lethal outcome was recently reported from a 70-year-old patient after preceding operation due to coronary heart disease and aortic aneurysm which was caused by transfusion of red blood cell concentrates. One of the blood donors was a 30-year-old man born in Cameroon who had left the country 10 years before and had ever since lived in Paris (near the International Airport) and later on in Switzerland. His last visit to his home country had been 6 years back at the time of the medical investigations. A possible cause of the infection of the donor was assumed to be airport malaria or persistence with partial immunity [57].

3.2 Immune Status (Resistance, Existing Immunity, Immune Response, Age, Exogenous Factors) [3, 4]

The course of malaria strongly depends on the degree of immunity of the infected individual. Complete sterile immunity is never reached, but merely partial immunity (semi-immunity). Immunity is proportional to the age, the cumulative number of malaria episodes and time spent continuously in a malaria endemic region.

Based on transplacentally transmitted maternal antibodies, newborns have a certain degree of immunity to malaria up to an age of around 4–6 months, whereby foetal haemoglobin inhibits maturing of the schizonts. Older children and young adults in regions with stable malaria and a high transmission rate (holoendemic or hyperendemic regions) possess the highest degree of immunity. Here, asymptomatic parasitaemia can often be found. In semi-immune individuals – polyclonal increase in IgM, IgG and IgA antibodies as well as specific T-lymphocytes can act cytotoxically against parasites or infected cells in the liver. The most important antigen in infections with *P. falciparum* is probably variable protein PfEMP-1. The acquisition of antibodies against a number of variant PfEMP-1 antigens seems to be of special significance for the immunity situation. Specific immunity is directed both against the species and against the respective subpopulation of the parasites.

Non-immune individuals only have the non-specific defence mechanisms, and the course of tropical malaria in such individuals is by far more severe than in semi-immune persons.

Congenital red blood cell anomalies also play an important role in immunity to malaria. In malaria endemic areas, hereditary diseases such as sickle cell anaemia, thalassaemia or glucose-6-phosphate dehydrogenase deficiency of the red blood cells can be found. In individuals with heterozygoty of HbAS (sickle cell gene carriers), there is a certain immunity against tropical malaria. This seems to be due to the inhibited growth of the parasites caused by strongly reduced oxygen content. Higher resistance can also be found in heterozygote α-thalassaemia. Glucose-6-phosphate dehydrogenase deficiency is also associated with reduced susceptibility to malaria in children and pregnant women and is caused by inhibition of growth of the parasites in red blood cells in which the latter enzyme is missing. Ovalocytosis, which occurs in South-East Asia, leads to lower parasitaemia in heterozygote carriers.

Red blood cells where the Duffy blood group antigen Fy^a is missing are partly resistant to *P. vivax*. This is how the very low incidence of malaria caused by *P. vivax* in West Africa can explained, where a high percentage of the population does not possess the Duffy blood group antigen Fy^a [58].

3.3 Severity and Course of the Disease

Untreated tropical malaria always has a fatal outcome in non-immune individuals who do not originate from an endemic area.

In a US study performed on transfusion associated malaria from the years 1963–1998 with a total of 93 cases, a lethality rate of 11% (10 cases) was observed [45]. Six of these patients had an infection with *P. falciparum*, 2 were infected with *P. vivax*, and 2 with *P. malariae*. The two patients with *P. vivax* died of their underlying disease.

Out of 5 patients with post-transfusion malaria in the UK [59], 1 died of cerebral tropical malaria and another *P. falciparum* infected patient died of multiple organ failure. In another study [60], lethality of transfusion associated malaria is indicated as 15%. No figures are available from Germany. The high lethality rate of transfusion associated malaria can be explained by the fact that most of the cases documented are cases of immunologically naïve recipients with a more or less serious underlying disease, or this rare route of infection was diagnosed too late.

The incubation period of transfusion associated malaria depends on the species and the number of transmitted parasites, and is between 10 and 60 days [43]. For *P. falciparum*, it is 10 days on average, for *P. vivax* 16 days, and for *P. malariae* 40 days [42]. Symptoms of transfusion associated malaria are many and varied. They include dizziness, vomiting, muscle pain, slight icterus, abdominal pain and diarrhoea. Usually, no fever periodicity can be found. In patients with serious underlying disease, above all immune suppression, transfusion associated malaria often displays a severe course with early cerebral involvement.

Table 2. Overview of products for malaria prophylaxis and stand-by treatment for malaria (modified in accordance with the recommendations from the *Deutsche Gesellschaft für Tropenmedizin und Internationale Gesundheit* (DTG, German Society for Tropical Medicine and International Health)

Product	Prophylaxis	Stand-by treatment
Chloroquine	300 mg chloroquine base weekly – for persons > 75 kg body weight: 450 mg weekly – 1 week before up to 4 weeks after a stay in a malaria area	600 mg base, 6 h after the beginning of treatment and 300 mg at a time 24 and 48 h after the beginning of treatment
Mefloquine	250 mg weekly, 1–3 weeks before up to 4 weeks after a stay in a malaria area	Initial dose 750 mg, after 6–8 h further 500 mg; if body weight >60 kg after another 6–8 h further 250 mg
Atovaquone-proguanil	250 mg/100 mg daily, 1–2 days before up to 7 day after a stay in a malaria area (adults with a body weight > 40 kg; maximal duration of stay 28 days)	1,000 mg / 400 mg as a single dose on 3 subsequent days if the body weight is > 40 kg
Doxycyclin	100 mg daily 1–2 days before and up to 4 weeks after a stay in a malaria area	not suitable
Artemether-lumefantrine	not suitable	80 mg / 480 mg initial dose after 8 h further 80 mg / 480 mg, then 2× daily 80 mg / 480 mg on days 2 and 3
Proguanil	200 mg daily	not suitable

3.4 Therapy and Prophylaxis

3.4.1 Vaccine Development

Although a variety of attempts have been made to develop effective malaria vaccines, practically no immunisation is commercially available yet [61]. In the past few years, malaria has been recognised as a global social problem, and intensive activities are underway to develop new vaccines, which is to a considerable extent the merit of the generous funding from the European Union and the Bill and Melinda Gates Foundation. The number of clinical trials is on the increase so that new vaccines can be expected in the next few years. The distribution of roles between humoral and cell-bound factors in effective immunity against plasmodia, however, is not understood [58].

The development work focuses on vaccines against *P. falciparum* and/or tropical malaria as the most dangerous form of malaria. Four stages of the life cycle of *P. falciparum* are eligible as targets of immune prophylaxis in humans:

- sporozoites after a blood meal or before infection of the hepatocytes,
- the merozoites after release from the liver or before invasion of the red blood cells,
- the merozoites during invasion of the red blood cells or replication in the red blood cells, and
- the gametocytes released from the red blood cells in the anopheles mosquito after the next blood meal [61].

The overwhelming majority of the development studies focus on potential vaccines which are intended to prevent the invasion in the red blood cells, and thus the massive replication of the parasites. It is expected that this would inhibit the clinical symptoms of malaria, and above all, the complications relating to these symptoms. If the red blood cell stage could be completely blocked, this would automatically break the infection chain which would also be significant from an epidemiological point of view. Strictly speaking, only vaccines against sporozoites preventing the invasion of the liver cell and leading to the killing of parasites should be considered as causal immune prophylaxis. Such variants, however, will probably not be a subject of discussion in the near future, due to the complexity of the parasites and the number of unresolved scientific problems. From the transfusion medicine point of view, we must bear in mind that, even if a donor is protected by a vaccination, it should not automatically be assumed that no transfusion related malaria will occur. What is more relevant here is to calculate this risk taking into account the epidemiological and infectiological background and the type of immune protection.

3.4.2 Prophylaxis and Therapy

Primary prevention of malaria is brought about by mosquito bite prevention. Other preventative measures include the reduction of the parasite reservoir in the population in malaria endemic regions, measures to eradicate vectors (removal of breeding grounds, use of larvicides, and insecticides), as well as measures to reduce contact with the vector.

Attempts have been made to immunise the *Anopheles* mosquito against the parasite in order to break the infection chain. Travellers to malaria endemic areas are still strongly recommended to undergo exposure prophylaxis, e.g. the use of repellents, wearing clothes that provide protection, sleeping under mosquito netting and staying in mosquito proof rooms. In addition, chemoprophylaxis is recommended depending on the destination and the type of travel. Depending on the resistance situation, the Deutsche Gesellschaft für Tropenmedizin und Internationale Gesundheit (DTG; German Society for Tropical Medicine and International Health) recommends chloroquine, mefloquine, atovaquone-proguanil, or, in some cases, doxycyclin for malaria prophylaxis (table 2) [62]. In this context, the distinction is made between continued chemopro-

Table 3. Treatment of malaria, modified in accordance with [63]

	Product	Dosage
Malaria tertiana Malaria quartana	chloroquine	10 mg base/kg body weight initial dose, followed by 5 mg base/kg body weight after 6, 24, and 48 h
	plus primaquine (only in case of tertian malaria)	0.5 mg base/kg body weight 1 × daily over a period of 14 days
Malaria tropica		
Non-complicated malaria	mefloquine	initially 750 mg base, followed by 500 mg base 6 h after the beginning of treatment and 250 mg base 12 h after the beginning of treatment (at >60 kg body weight)
	or atovaquone-proguanil	1,000 mg/day atovaquone, 400 mg/day proguanil 1 × daily over a period of 3 days
	or arthemether-lumefantrine	80 mg / 480 mg arthemether-lumefantrine initial dose, followed by 80 mg / 480 mg arthemether-lumefantrine after 8 h, 2 × 80 mg / 480 mg arthemether-lumefantrine on day 2, 2 × 80 mg / 480 mg arthemether-lumefantrine on day 3
Complicated forms	quinine salt (quinine-dihydrochloride)	3 × 10 mg p.inf./kg body weight/day for 7–10 days
	plus doxycyclin	3 mg/kg body weight/day for 7 days

phylaxis (which as a rule begins one week or day before travelling to the malaria area and continues up to 4 weeks or 1 week after leaving the malaria area, depending on the situation), and the stand-by treatment with the appropriate medicine (mefloquine, atovaquone-proguanil, artemeter-lumefantrine). The use of halofantrin for malaria prophylaxis was stopped in Germany many years ago because of its risk of cardiac adverse effects.

In pregnant women, the use of a combination of chloroquine and proguanil is possible as from the second trimester. For children primarily consistent exposure prophylaxis is recommended. Prophylaxis with atovaquone-proguanil is possible for children with a body weight ≥11 kg while mefloquine can be used in children with a body weight over 5 kg. Doxycyclin must not be used in children under 8 years of age. However, chemoprophylaxis does not guarantee that the patient will be free from malaria, but will reduce the risk significantly.

Because of the world-wide occurrence of e.g. chloroquine and sulfadoxin-pyrimethamine resistent isolates of *P. falciparum*, meloqine, atovaquone-proguanil, or arthemether-lumefanine are now the treatments of choice in non-complicated tropical malaria. Treatment of serious tropical malaria (with CNS involvement, acute kidney failure or other organ complications) should be performed under intensive-ward conditions at all means. Treatment with medicinal products usually consists of parenteral administration of quinine in combination with doxycyclin.

Due to the still relatively favourable resistance situation, chloroquine is still the treatment of choice in tertian malaria. After completion of the chloroquine course of treatment, completion treatment with primaquine which is efficacious against hypnozoites of *P. vivax* and *P. ovale* should follow. Quartan malaria should also be treated with chloroquine. Subsequent treatment with primaquine is not required (table 3).

The same principles as for general malaria treatment apply for treatment of transfusion associated malaria, except that in infections caused by *P. vivax* and *P. ovale*, no subsequent treatment with primaquine is required because a liver cycle, i.e. hypnozoites, is not involved in this type of infection (cf. 1.2. 'Infection and Infectious Disease'). In this type of infection, too, it is early start of treatment which is decisive for the outcome. Prophylaxis is currently possible only by strict exclusion criteria for donors, in combination with serological and/or molecular biology screening (NAT) if required. A vaccination which confers protection is so far not available.

3.5 Transmissibility

A risk of transmissibility of malaria by transfusion exists because of the primary presence of parasites in red blood cells, above all in transfusion of whole blood or red blood cells. However, there is also a risk of transmission with platelet concentrates [64], leucocyte concentrates [65], and even fresh frozen plasma; the latter has only been observed with the administration of fresh frozen plasma within one day following collection [66]. Three infections with malaria were described due to non-observance of the basic principles of hygiene [67] or needle perforation injury [10, 68], and three other transfusion associated cases of malaria recently were described in Canada [46]. These give rise to the assumption that even low numbers of infected red blood cells can cause malaria infection.

Due to the long survival times in the human organism (e.g. *P. malariae*), a donor can still have malaria parasites in the blood after years and can cause an infection in the recipient. In general, *P. vivax* and *P. ovale* rarely persist longer than 3 years while *P. falciparum* persists about 1–2 years. The longest intervals between blood donation and malaria exposure of the donor in the past were 13 years in the case of a *P. falciparum* infection [69], 27 years for a *P. vivax* infection [69] and 7 years in a *P. ovale* infection [70]. For *P. malariae*, considerably longer time intervals have been described – in an extreme case – up to 50 years or even more.

3.6 Frequency of Administration, Type and Amount of Blood Products

For plasma derivatives, contamination with plasmodia can be excluded thanks to the manufacturing procedure.
In principle, transmission is possible with only one single product of a non-pathogen-inactivated blood component since all products show residual red blood cell contents.

4 Blood Products

4.1 Infectious Load of the Starting Material and Test Methods

Most donors involved in the cases of transfusion associated malaria are semi-immune individuals with low parasitaemia in the blood. As is known from previous studies, plasmodia can survive in blood reserves for at least 10–12 days, possibly longer [43, 71]. Apparently, the minimum infective dose in humans is 10 parasites (for *P. vivax*) [65]. In the case of assumed (low) parasitaemia in the donor of 1–2 parasites per µl blood, however, as many as 250,000–500,000 parasites would be transmitted if 250 ml red blood cell concentrate was donated. Methods for detecting a potentially infected donation which would have to contain as few as 10 parasites, would have to be able to detect as little as 0.00004 parasites per µl blood [43, 72]. This degree of sensitivity is not even achieved with the NAT method.
The test methods usually applied for malaria such as microscopic examination of 'thick drop' or blood smear in accordance with Giemsa (sensitivity for both methods between 5–500 parasites/µl blood) are not suitable for donor screening because of the usually very low parasitaemia of the donor. Antigen tests based on the detection of HRP/2 or pLDH, too, only detect between 100 and 1,000 parasites/µl blood. Compared with that, NAT methods display higher sensitivity [73, 74]. In a study [75], as little as 0.004 parasites/µl blood could be detected in potentially malaria exposed donors. However, NAT is not able to detect all potentially infectious donors, even at this high sensitivity.

Targeted serological examination of donors returning from malaria endemic areas has been successfully performed in France since 1983. After a deferral period of 4 months, donors are tested using the IFAT. If the result is negative, the donor can be re-admitted. No transfusion associated case of malaria became known in France between 1984 and 2002 [76]. Disadvantages of the IFAT however, include its limitation to antibodies against *P. falciparum* with low cross-reactivity against the other plasmodia species, high workload in the laboratory and its poor reproducibility due to the subjective assessibility of the test method. Enzyme immunoassays have recently been developed, partly also with recombinant antigens [44, 72]. Thus, a combination of donor interviewing and testing with enzyme immunoassays with recombinant antigen is currently recommended in England for reducing the malaria risk after blood transfusion.

4.2 Methods for Removal and Inactivation of the Infectious Agent

For plasma derivatives, transmission of malaria can be excluded thanks to the manufacturing procedure. Therefore, no cases of malaria due transmission of fractionated plasma products have so far been described.

4.3 Feasibility and Validation of Procedures for Removal/ Inactivation of the Infectious Agent

Some papers have recently been published on the inactivation of plasmodia in blood components by means of different methods (gamma irradiation, photochemical and photodynamic inactivation) [77–81], but without being able to establish whether these methods can be considered as sufficiently reliable and without being considered as routine procedures up to now. In contrast to that, the INTERCEPT system for pathogen inactivation in platelet concentrates and plasma for transfusion is currently beginning to become established in the blood bank routine of some countries. This photochemical method in which the photoactive compound amotosalen HCI is added to the blood components which are at the same time irradiated with long-wavelength UV light (UVA) has proven to be effective for *P. falciparum* by showing a pathogen reduction of >6 log levels [82].

5 Assessment

With by far more than 1 billion people affected, malaria, which occurs in the world's tropical and subtropical areas, is one of the most significant parasitary infections in humans. The protozoae, which are transmitted by the sting of the female *Anopheles* mosquito, (*P. falciparum*, *P. vivax*, *P. ovale* and *P.*

malariae) cause different forms of malaria in humans. Tropical malaria which is caused by *P. falciparum* is the infection mainly responsible for the 1–3 million deaths occurring per year world-wide, and out of which more than half are African children.

In Germany, the number of malaria infections reported in the past few years varies between around 600 and 1,000 cases per year. These are caused by travelling, very rarely by importing infected *Anopheles* (airport malaria) and in very isolated cases by autochthonous infections.

Depending on the resistance situation, medicinal products such as chloroquine, mefloquine, atovaquone-proquanil, or doxycyclin are used for chemical malaria prophylaxis. Donors are deferred independently from such a malaria prophylaxis. No vaccination is currently commercially available which confers protection against malaria.

During the period up to 1965, altogether 12 cases of transfusion associated malaria became known in Germany. Three reports on malaria following blood transfusions in Germany are available in the literature from a more recent time. Since the Paul Ehrlich Institute became responsible for blood products (1994), one single case of transfusion associated malaria was reported from 1997.

To prevent transfusion associated malaria, persons are excluded from donating blood after medically documented cure from malaria for a period of 4 years. In addition, persons who were born or grew up or temporarily had there centre of life in a malaria endemic area are excluded from donating blood for 4 years following their stay in the endemic area. Returnees from a malaria endemic area are currently deferred from donating blood for a minimum of 6 months following their last stay in a malaria endemic area. If only plasma for fractionation is donated, no exclusion from the donation based on a malaria risk is required.

Altogether, this risk of transfusion associated malaria is considered as low in Germany. A high standard of safety is guaranteed thanks to the thorough investigation of the history of the patient and donor selection as well as the exclusion criteria used for returnees from tropical areas. New epidemiological data for Germany show that with the current referral period of 6 months after travels to endemic areas not all plasmodia infections are detected, especially infections with *P. vivax* or *P. ovale*, since in 15% of the cases reported, symptoms occur only after that period. An extension of the deferral period to 12 months would include nearly 99% of the malaria infections. An argument against an extension of the deferral period and the loss of donors relating to this would be the fact that there has so far been no case of transmission since the introduction of the 6-month regulation. If the epidemiological situation changes, however, the deferral period will have to be reviewed and adjusted.

Even though testing of blood donors for malaria antibodies or the presence of plasmodium antigens or for plasmodium genome by means of the NAT method is laid down in the 'Richtlinien zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Hämotherapie)' (Guidelines for the collection of blood and components from blood, as well as for the use of blood products (haemotherapy)) [39] for donors before re-admittance as donor after the 4-year deferral, no tests are commercially available guaranteeing the detection of antibodies against all four human pathogenic *Plasmodium* spp., which would, however, be imperative for diagnostics with informational value. The inactivation of plasmodia in blood components, which is feasible in principle, should not be required as a general method for Germany because of the low malaria prevalence. Concerning this issue as well as concerning the development of a suitable vaccine, extensive research is certainly required in order to optimise the safety for recipients of blood and blood products regarding malaria.

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