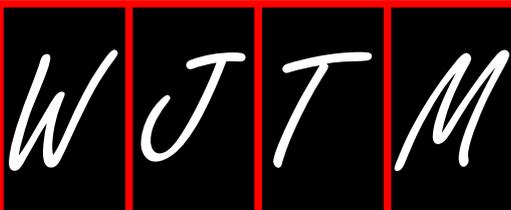


World Journal of *Translational Medicine*

World J Transl Med 2012 August 12; 1(2): 4-19





World Journal of Translational Medicine

A peer-reviewed, online, open-access journal of translational medicine

Editorial Board

2012-2016

The World Journal of Translational Medicine Editorial Board consists of 103 members, representing a team of worldwide experts in translational medicine. They are from 27 countries, including Argentina (1), Australia (2), Belgium (1), Canada (2), China (1), Czech (2), France (7), Germany (9), Greece (2), India (3), Iran (1), Israel (2), Italy (17), Japan (4), Malaysia (1), Mexico (2), Netherlands (1), Peru (1), Poland (1), South Africa (1), Spain (2), Sweden (1), Turkey (4), United Arab Emirates (1), United Kingdom (7), United States (26), and Venezuela (1).

EDITOR-IN-CHIEF

Alfonso Dueñas-González, *Mexico*
Ruggero Ridolfi, *Meldola*

GUEST EDITORIAL BOARD MEMBERS

Mu-Rong Chao, *Taichung*

MEMBERS OF THE EDITORIAL BOARD



Argentina

Daniel P Cardinali, *Buenos Aires*



Australia

Moyez Jiwa, *Perth*
Jagat R Kanwar, *Geelong*



Belgium

Olivier Bruyère, *Liège*



Canada

Tarek El-Bialy, *Edmonton*
Tatsuya Kin, *Edmonton*



Czech Republic

Martin Huser, *Brno*
Kamil Kuca, *Hradec Kralove*



France

Nadia Alfaidy, *Grenoble*

Alain E Braillon, *Amiens*
Bruno Dubois, *Paris*
Philippe Gervois, *Lille*
Gilles Gosselin, *Montpellier*
Cyril Goudet, *Montpellier*
Béchrir Jarraya, *Paris*



Germany

Heike Allgayer, *Mannheim*
Thomas Bock, *Berlin*
Stefan R Bornstein, *Dresden*
Guo Cheng, *Dortmund*
Hassan Dihazi, *Goettingen*
Thomas Efferth, *Mainz*
Benedikt Fritzsching, *Heidelberg*
Axel M Gressner, *Aachen*
Wolfgang E Jelkmann, *Luebeck*



Greece

Evangelos C Alexopoulos, *Athens*
Demosthenes Bouros, *Alexandroupolis*



India

Ritesh Agarwal, *Chandigarh*
Sudeep Gupta, *Mumbai*
Anisur R Khuda-Bukhsh, *Kalyani*



Iran

Leila Azadbakht, *Isfahan*



Israel

Zeev Blumenfeld, *Haifa*

Oren Froy, *Rehovot*



Italy

Walter Arancio, *Palermo*
Luca Arcaini, *Pavia*
Giuseppe Argenziano, *Naples*
Elisabetta Baldi, *Florence*
Saverio Bettuzzi, *Parma*
Giuseppe Biondi-Zoccai, *Modena*
Giampaolo Bresci, *Pisa*
Giuseppe Maurizio Campo, *Messina*
Andrea Cavalli, *Bologna*
Massimo Chello, *Rome*
Silvia Deaglio, *Turin*
Alfio Ferlito, *Udine*
Daniela Galimberti, *Milan*
Giuseppe Giannini, *Pomezia*
Fabio Grizzi, *Rozzano*
Pietro Invernizzi, *Rozzano*



Japan

Takaaki Arigami, *Kagoshima*
Katsuya Dezaki, *Tochigi*
Akira Hokama, *Okinawa*
Kenji Kabashima, *Kyoto*



Malaysia

Azarisman MS Shah, *Kuantan*



Mexico

Gabriel Gutiérrez-Ospina, *Mexico*



Netherlands

Pim MW Janssens, *Arnhem*



Peru

Luis Huicho, *Lima*



Poland

Krzysztof Ksiazek, *Poznan*



South Africa

Marlon Eugene Cerf, *Cape Town*



Spain

Pablo Avanzas, *Oviedo*
Alberto Ortiz, *Madrid*



Sweden

Stefan Karlsson, *Lund*



Turkey

Özkan Ateş, *Tekirdağ*
Johann Bauer, *Bursa*
Gulbeyaz Can, *Istanbul*
Suleyman Kaplan, *Samsun*



United Arab Emirates

Taleb H Al-Tel, *Sharjah*



United Kingdom

Sabine Bahn, *Cambridge*
Dominique Bonnet, *London*
Darren R Brooks, *Salford*
Vincent P Collins, *Cambridge*
William Davies, *Cardiff*
Mohamed El-Tanani, *Belfast*
Christopher L Jackson, *Bristol*



United States

Hossam M Ashour, *Detroit*
Iris Asllani, *New York*

Vineet Bhandari, *New Haven*
Guoyin Bing, *Lexington*
David L Brown, *Stony Brook*
Manuel F Casanova, *Louisville*
Amy Suzon Chappell, *Indianapolis*
Georgia Zhuo Chen, *Atlanta*
Xiaozhuo Chen, *Athens*
Undurti N Das, *Shaker Heights*
Bingliang Fang, *Houston*
Yubin Ge, *Detroit*
Shannon S Glaser, *Temple*
Ajay Goel, *Dallas*
Ahmet Gokce, *New Orleans*
Mitchell H Grayson, *Milwaukee*
Kuzhuvelil B Harikumar, *Richmond*
David W Hein, *Louisville*
Zdenek Hel, *Birmingham*
Matthew Owen Howard, *Chapel Hill*
Shile Huang, *Shreveport*
Toshiaki Kawakami, *La Jolla*
Mark S Kindy, *Charleston*
Siva Kumar Kolluri, *Corvallis*
Irwin J Russell, *San Antonio*
Georg F Weber, *Cincinnati*



Venezuela

Leonor Chacin-Bonilla, *Maracaibo*



REVIEW

- 4 Recent advances and perspectives on tropical diseases: Malaria
Micale N

ACKNOWLEDGMENTS I Acknowledgments to reviewers of *World Journal of Translational Medicine*

APPENDIX I Meetings
I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Translational Medicine*, Ruggiero Ridolfi, MD, Director, Immunotherapy and Somatic Cell Therapy Unit, Romagna Cancer Institute, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Via Piero Maroncelli, 40, 47014 Meldola, Italy

AIM AND SCOPE *World Journal of Translational Medicine (World J Transl Med, WJTM, online ISSN 2220-6132, DOI: 10.5528)* is a bimonthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 103 experts in translational medicine from 27 countries.

The aim of *WJTM* is to report rapidly new theories, methods and techniques for prevention, diagnosis, treatment, rehabilitation and nursing in the field of translational medicine, focusing on information derived from human experimentation so as to optimize the communication between basic and clinical science. *WJTM* covers animal and human studies, applied physical sciences, behavior, bioengineering, biomarkers, cancer, cardiovascular disease, cell culture, chemical genomics/drug discovery, data mining, drug delivery, gene therapy/regenerative medicine, imaging, immunology/vaccines, infectious diseases, medical informatics, medical nanotechnology, metabolism/diabetes/obesity, neuroscience/neurology/psychiatry, other interdisciplinary approaches to medicine, physics, policy, toxicology and pharmacokinetics, and translational medicine-related traditional medicine, integrated Chinese and Western medicine. The journal also publishes original articles and reviews that report the results of translational medicine-related applied and basic research in fields such as immunology, physiopathology, cell biology, pharmacology, medical genetics, and pharmacology of Chinese herbs.

FLYLEAF I-II Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Shuai Ma*
Responsible Electronic Editor: *Li Xiong*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Ling-Ling Wen*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Translational Medicine

ISSN
ISSN 2220-6132 (online)

LAUNCH DATE
June 12, 2012

FREQUENCY
Bimonthly

EDITING
Editorial Board of *World Journal of Translational Medicine*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: wjtm@wjnet.com
<http://www.wjnet.com>

EDITOR-IN-CHIEF
Alfonso Dueñas-Gonzalez, MD, PhD, Unit of Biomedical Research on Cancer, Instituto de Investigaciones Biomédicas, UNAM, Instituto Nacional de

Cancerología. Primer piso, edificio de Investigación, San Fernando 22, Itzapán 14080, Mexico

Ruggiero Ridolfi, MD, Director, Immunotherapy and Somatic Cell Therapy Unit, Romagna Cancer Institute, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Via Piero Maroncelli, 40 - 47014 Meldola, Italy

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Translational Medicine
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: wjtm@wjnet.com
<http://www.wjnet.com>

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046

E-mail: bpg@baishideng.com
<http://www.wjnet.com>

PUBLICATION DATE
August 12, 2012

COPYRIGHT
© 2012 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjnet.com/2220-6132/g_info_20100722180909.htm.

ONLINE SUBMISSION
<http://www.wjnet.com/2220-6132office/>

Recent advances and perspectives on tropical diseases: Malaria

Nicola Micale

Nicola Micale, Department of Drug Sciences and Health Products, University of Messina, 98168 Messina, Italy

Author contributions: Micale N solely contributed to this paper.
Correspondence to: Nicola Micale, PhD, Assistant Professor, Department of Drug Sciences and Health Products, University of Messina, 98168 Messina, Italy. nmicale@unime.it
Telephone: +39-90-6766419 Fax: +39-90-6766402
Received: June 22, 2012 Revised: June 31, 2012
Accepted: July 25, 2012
Published online: August 12, 2012

Peer reviewer: Mark S Kindy, Professor, Department of Neurosciences, Medical University of South Carolina, 114 Doughty Street, Room 503, Charleston, SC 29425, United States

Micale N. Recent advances and perspectives on tropical diseases: Malaria. *World J Transl Med* 2012; 1(2): 4-19 Available from: URL: <http://www.wjgnet.com/2220-6132/full/v1/i2/4.htm> DOI: <http://dx.doi.org/10.5528/wjtm.v1.i2.4>

Abstract

Malaria remains a major health problem in the world. It is a neglected disease because it occurs almost exclusively in poor developing countries, which offer negligible marketable and profitable opportunities. Malaria (together with Tuberculosis), is responsible for an unprecedented global health crisis with devastating effects in developing countries. The 2011 World Malaria Report indicated that 106 countries showed endemic malaria. Malaria control depends mainly on drug treatment, which is increasingly difficult due to the spread of drug resistant parasites and requires expensive drug combinations. Part of the inability to combat this disease is attributed to an incomplete understanding of its pathogenesis and pathophysiology. Improving the knowledge of the underlying pathogenic mechanisms of malaria transmission and of the exclusive metabolic pathways of the parasites (protozoa of the genus *Plasmodium*), should promote efficient treatment of disease and help the identification of novel targets for potential therapeutic interventions. Moreover, the elucidation of determinants involved in the spread of malaria will provide important information for efficient planning of strategies for targeted control.

© 2012 Baishideng. All rights reserved.

Key words: Malaria; Anopheline mosquitoes; Plasmodium parasites

EPIDEMIOLOGY

Malaria remains one of the most deadly parasitic diseases with an estimated 216 million clinical cases and about 655 000 deaths annually. Currently 86% of deaths happen in sub-Saharan Africa, especially in children under 5 years of age^[1].

Malaria burden is caused by *Plasmodium* (*P.*) parasite, transmitted through the bite of infected female anopheline mosquitoes. Four species are able to infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. Among these, *P. falciparum* is the most lethal species and, together with *P. vivax* accounts for more than 95% of malaria cases in the world^[2]. More recently, cases of infections in humans due to *P. knowlesi*, a malaria parasite normally hosted by “kra” monkey, have been reported in South East Asia^[3].

There is no completely satisfactory epidemiological classification of malaria. It is possible to distinguish between a high and stable malaria transmission, and a low and unstable malaria transmission strictly dependent on mosquito species (the vector of the disease), geographic and climatic factors^[4]. The most common parameter used for the measurement of malaria transmission is the number of infective mosquito bite per year. Stable malaria transmission is widespread in South of the Sahara where populations are continuously challenged with malaria inoculations from childhood onwards. This continuous exposure to the infection leads to a partial immunity in adults. Unstable malaria transmission is typical of Asia and Latin America, where the populations pos-

ness no immunity because of the low rate of exposure to malaria^[4].

STRATEGY OF CONTROL

In the absence of an effective vaccine, malaria control relies on a dual approach: primarily in mosquito control and secondarily in reducing the parasite reservoir in the population. World Health Organization (WHO) recommends preventive measures: (1) the use against malaria parasites of insecticide treated nets and indoor residual spraying with insecticide; and (2) the employment against malaria parasites of an intermittent preventive treatment in travellers in endemic regions, and in vulnerable populations in high transmission areas.

For the treatment of uncomplicated malaria WHO recommended from 2001 the adoption of artemisinin-based combination therapies (ACTs). These therapies are now based on the combination of an artemisinin derivative together with a blood schizonticidal drug; the most commonly used are combinations of artesunate-sulfadoxine/pyrimethamine, artesunate-amodiaquine, artesunate-mefloquine, and artemether-lumefantrine^[5]. In the updated treatment guidelines, WHO strongly recommends the dihydroartemisinin-piperaquine combined therapy as the most effective ACT. In contrast, the treatment of severe malaria is normally based on the administration of parenteral antimalarial drugs, which must be immediate, appropriate and effective. Intravenous artesunate is recommended as the first choice treatment for severe malaria. Quinine constitutes a valid alternative when parenteral artesunate is not available. Following initial parenteral treatment, it is necessary to continue and complete treatment with a full course of an effective ACT.

Unfortunately, malaria parasites develop resistance to almost every known antimalarial drug including artemisinin derivatives. For this reason, new antimalarial drugs synthesized by medicinal chemists are needed, targeting metabolic pathways of the parasite and able to by-pass resistance mechanisms enacted by the parasite^[6,7].

PATHOPHYSIOLOGY

The pathophysiology of malaria represents one of the most interesting aspects of this infectious disease although knowledge of the complex biochemical mechanisms involved to date remains incomplete.

In areas where malaria is highly endemic, malarious patients can experience an asymptomatic parasitemia mainly due to immunological phenomena or manifest non-specific symptoms with a gradual or a fulminant course. Generally malaria is defined as an acute febrile illness whose symptoms resemble those of common viral infections (e.g., malaise, dizziness, myalgia, nausea, vomiting, and diarrhea). This may lead to a delay in diagnosis which is not often available and reliable, particularly in rural zones. In addition to fever, typical physical signs of

malaria infection include chills, headache, tachycardia, jaundice, pallor, orthostatic hypotension, hepatomegaly, and splenomegaly. Severe malaria may lead to permanent cerebral damage, acute anemia, respiratory distress and metabolic complications (especially acidosis and hypoglycemia), which may develop rapidly and progress to death within hours or days^[8].

All clinical manifestations of malaria are related to the intra-erythrocytic growth phase of the parasite^[9]. The so-called “primary attack” is usually atypical and may resemble any febrile illness. It occurs after a variable prepatent period, which is the time that elapses between infected mosquito bite and the appearance of symptoms. During this prepatent period, *Plasmodia* (injected in form of sporozoites) undergo schizogony in the liver (first part of asexual cycle), evolving into schizonts, then into encapsulated merozoites. Following the rupture of the hepatocytes, merozoites migrate through the bloodstream into the erythrocytes where they undergo another schizogony cycle (second part of asexual cycle)^[10]. Newly formed merozoites rupture the host cell and invade new erythrocytes. It is now well known that *P. falciparum* proteases of at least two classes (serine and cysteine) play a crucial role in host cell invasion and egress processes^[11], and specific inhibitors are currently the subject of extensive research into their role as possible drug candidates^[12].

The lysis of erythrocytes is responsible for fever and related symptoms, while during the prepatent period some individuals may show no symptoms or only vague signs of illness such as headache, aches and pains, nausea. When the infection stabilizes the individual manifests “short term relapses” of symptoms at regular intervals of 48-72 h, depending on the *P. species*. These are intervals between two consecutive erythrocyte ruptures, corresponding to the erythrocytic phase of malaria parasites. Sporozoites of *P. vivax* and *P. ovale* are able to remain in the liver as dormant hypnozoites, capable of causing “long term relapses” after an average of 2-3 mo, sometimes even after years from the initial infection. In cases of *P. falciparum* and *P. malariae* infection, relapses from the liver do not occur. However, the blood infection may remain chronic and, if untreated, may remain chronic for years in case of *P. falciparum* and decades in case of *P. malariae*. Some of the merozoites do not undergo sporogony but develop into sexual stages microgametocytes (male) and macrogametocytes (female) instead, degenerating within 6-12 h if they are not ingested via another mosquito bite. Finally, the parasite completes its life cycle inside the mosquito midgut where a complex process of differentiation, growth and fertilization of gametocytes takes place, leading to the formation of diploid zygotes. These zygotes further differentiate into mobile ookinetes that move to the midgut surface and mature into oocysts. Thousands of sporozoites are formed within the oocyst, thereby making their way to the salivary glands. The infected mosquito is now able to transmit the infection during its next blood meal. The

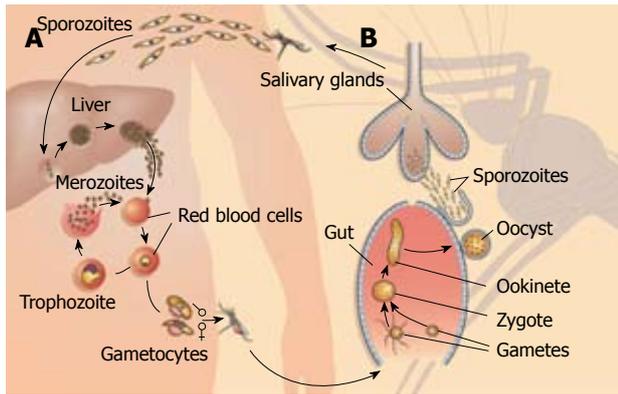


Figure 1 Plasmodium life cycle. A: In the human host, infected female *Anopheles* mosquitoes inject the sporozoite form of the parasite during a blood meal. Sporozoites reach the liver through the bloodstream, where they proliferate asexually, and then, as merozoites, invade red blood cells, evolve and eventually generate male and female gametocytes which are transmitted back to the mosquito; B: In the mosquito vector, gametocytes fuse to form zygotes and differentiate to form oocysts that duly divide to create sporozoites in the mosquito midgut. These migrate to the salivary glands, where the cycle of infection starts again.

entire *Plasmodium* life cycle is depicted in Figure 1.

Cerebral malaria is the most serious complication of malaria, and it is caused almost exclusively by *P. falciparum* as a consequence of the clogging of the cerebral micro-circulation. Only a few cases of severe *P. vivax* malaria in adults have been reported to date^[13]. In *P. falciparum* malaria, the infected red blood cells (RBCs) develop knobs on their surfaces, which being sticky result in increased cytoadhesion and rosette formation, particularly in cerebral vessels. The infected RBCs may also adhere to the endothelium of capillaries and venules, further blocking the blood flow. By means of this immunological strategy, the parasite remains within the vascular compartment and avoids circulating through the spleen. The increased cytoadherence and clumping of uninfected RBCs together with parasitized RBCs culminates in damage to vital organs like brain, kidneys, lungs, liver, and gastrointestinal tract, leading to the various potentially fatal complications of *P. falciparum* malaria^[14]. Obstruction to the cerebral microcirculation results also in hypoxia and increased lactate production due to anaerobic glycolysis. The process of adhesion of infected erythrocytes in various tissues is called “sequestration”, and is thought to be the result of interaction between parasite-encoded variant surface antigens on the outer membrane of infected erythrocytes and a range of host receptors. The process is mediated by the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) which is expressed on the surface of infected erythrocytes and anchored into the knobs, thus representing a key virulence factor for this species of human malarial parasite^[15].

The rupture of RBCs by merozoites releases certain factors and toxins, which in turn induce the release of cytokines such as tumor necrosis factor (TNF) and interleukin-1 from macrophages, resulting in chills and high-grade fever. The tendency of erythrocytes to adhere to

blood vessels is also thought to be related to excessively high levels of TNF. The latter is in turn a potent inducer of nitric oxide (NO) synthase type 2 and a role for NO in the pathogenesis of cerebral malaria has, therefore, been suggested^[16]. The most sound hypothesis is that NO helps the body defend itself against malaria (either killing parasites or interfering with their ability to multiply), and that NO levels might even be a key factor in determining whether an individual will get a milder form of the disease or a life-threatening form^[17]. Likewise, there is evidence that an excessive production of NO may contribute to the brain damage caused by strokes and to the life-threatening plunge in blood pressure, known as septic shock, that occurs in some infections.

Anstey *et al*^[17] demonstrated controversial outcomes in a study conducted on a sample of Tanzanian children (with or without parasitemia). The very sickest children with cerebral malaria had the lowest levels of NO, and the highest levels were found in the children who remained healthy despite being infected with malaria organisms. The very sickest children did have high levels of TNF, and the researchers expected this to also lead to high NO levels, as previous studies had predicted. They hypothesized that the low levels of NO found in the sickest children probably occurred because the children had unusually high levels of interleukin-10, a substance made by the immune system that suppresses NO.

Overall, the immune system of the person plays a crucial role in the clinical response to infection and transmission of malaria. Humoral antibodies to sporozoites, intrahepatic parasites, merozoites, malaria toxins, parasite antigens on infected RBCs, intraerythrocytic parasites, and, within the mosquito, to parasite fertilization, have recently been identified, suggesting that cell-mediated immunity plays a role in liver and RBC invasion and parasite development. However, they may provide a form of non-sterile and age-dependent immunity termed “premunition”, which has to be maintained by almost continuous exposure to the parasites, another unique feature of this infectious disease. Individuals in malarious areas may gradually evolve from a state of immunity against clinical malaria to a state of partial immunity against the infection. Continuous exposure may be attained due to the long-term survival of parasites in the host and/or by frequent re-infections^[18].

Pregnant women constitute a notable exception to this, since they are highly vulnerable to infection due to general impaired immunity, particularly during a first pregnancy. The phenomenon of the slow acquisition of immunity and the sudden reappearance of susceptibility during pregnancy are strictly interrelated. In women during pregnancy, the placenta expresses a new receptor that was previously unavailable for infected RBC adherence^[19-21]. This receptor has been identified as a uniquely low-sulfated chondroitin sulfate proteoglycan (CSPG) located mainly in the intervillous space and to a lesser extent on the surface of the syncytiotrophoblast cells^[22,23]. It has been demonstrated that CSPG has a minimum

motif for optimal infected RBC binding located in its polysaccharide part. This binding site is a dodecasaccharide called “sulfated glycosamino-glycan chondroitin sulfate A (CSA)” and it is composed of a chain of alternating sugars (n-galactosamine and glucuronic acid) in which sulfation of the n-galactosamine moieties occurs at carbon 4 (CSA is therefore also known as chondroitin-4-sulfate)^[24-27]. Infected RBCs are tethered to the placenta surface through interactions between PfEMP1 domains and CSA, leading to inflammation and blockage of blood flow to the developing fetus. In this situation the host immune system is unable to detect and destroy infected RBCs in the spleen. Accumulation of infected RBCs in the placenta engenders monocyte/macrophage immune response, which in turn produce proinflammatory mediators^[28]. This is associated with all clinical conditions of placental malaria, including, maternal anemia, low birth weight of babies, premature delivery, spontaneous abortion, stillbirth, and can lead to the death of mother and child^[29-32]. Anti-adhesion antibodies from pregnant women (except primigravidas) may offer only limited and short-duration protection to the newborn^[33]. In rural areas with intense malaria transmission, approximately 30% (depending on the season) of infants at three months of age are infected by *P. species* and may develop their own partially protective immunity. This natural immunity may display a spectrum of anti-parasite activity and be effective in limiting parasite infectivity and parasite replication, thus having an impact on actual parasite burden in the human host. This may also limit infectivity of gametocytes in the mosquitoes and subsequent transmission to other hosts^[34].

While gradual acquisition of natural immunity is indisputable, the mechanism of stage-specific partially protective immunity in infants, adults and during pregnancy remain rather elusive^[34], raising questions around the efficacy of a long-term candidate vaccine that could provide only partial immunity. Understanding the mechanisms of immune response and identification of specific immunologic determinants will be fundamental for designing and developing promising vaccine candidates, including multistage vaccines. More likely, an efficacious fight against malaria will depend upon a combination of multistage vaccines with other malaria control options, including effective chemotherapy and anti-vector programs limiting host-vector interactions.

DETERMINANTS OF MALARIA

The progression of malaria and the toll of victims in communities and countries depend on several intrinsic and extrinsic determinants which often combine in a negative way. Of the intrinsic determinants, host (human) immunity, virulence of parasite species, anopheline longevity and avidity for humans have the greatest impact on the malaria burden^[35], and will be herein discussed in details. Among extrinsic determinants, climate (mainly rainfall), human activities, vector ecology and behaviour,

political and economic (poverty) conditions as well as effectiveness of control and prevention efforts are the most important.

Host

The role of host as a medium for parasite development is very complex and susceptibility to infection and severity of illness of human populations exposed to malaria infection may be influenced by other intrinsic factors (e.g., age, genetic disposition). Host immunity is a crucial and poorly understood factor for survival of people infected with the malaria parasite^[36]. This is particularly true with respect to the *P. falciparum* parasite which causes severe malaria, wherein many pathological processes such as hemostatic dysfunction, sequestration, systemic inflammation and neuronal damage are implicated^[37,38].

Sickle cell anemia provides the best example of a change in hemoglobin structure that impairs malaria parasite growth and development. Sickle cell consists in an inherited alteration of hemoglobin in which RBCs assume an abnormal, rigid, sickle shape. Some people who have the sickle cell trait (commonly written HbAS) inherit a normal hemoglobin A (HbA) gene from one parent and an abnormal hemoglobin sickle (HbS) gene from the other. These individuals are resistant to malaria since sickle RBCs are not conducive to the parasites^[39]. HbAS provides neither absolute protection nor invulnerability to malaria. Unlike people with sickle cell disease (who have mainly HbS) and people with normal hemoglobin genotype (HbAA), individuals (and particularly children) with HbAS and infected with *P. falciparum* are generally able to survive their initial acute malarial attacks. They are also referred to as “healthy carriers” because have neither symptoms and nor sickle-cell disease or sickle-cell anemia, although they carry one of the genes that cause the disease^[40]. This genetic selective scenario is termed “balanced polymorphism”, a concept strengthened by the observation that the heterozygous (HbAS) trait is more prevalent in regions of endemic malaria^[41]. Other genetic defects related to hemoglobin structure that confer protection against malaria include hemoglobin C (HbC), hemoglobin E (HbE), α^+ thalassemia, and β -thalassemia^[42]. HbS, HbC and HbE arise from a single point mutation of the gene HBB which encodes β -globin chains: Glu→Val at codon 6 for HbS, Gln→Lys for HbC and Glu→Lys for HbE^[43]. Both HbC-heterozygotes and HbC-homozygotes are protected against severe malaria with the latter being to a greater extent^[44-47], whereas a reduced parasite invasion by *P. falciparum* has been observed only for HbE-heterozygotes^[41,48]. In contrast, α^+ Thalassemia, arises from the disruption of only one of two identical genes encoding α -globin chains (HBA1 and HBA2, located on different chromosomes). In this particular condition, homozygous individuals are still able to produce α -globin and are only mildly anemic^[49].

Erythrocyte-membrane proteins represent other key factors of malaria progress since they are extensively

involved in the parasite invasion process and host-defensive mechanisms. Ovalocytosis and GYPs-deficit (the genes encoding glyophorin A, B and C) are among significant hereditary anomalies^[50,51]. The former, common in parts of Southeast Asia, consists in a deficit of an anion-exchange protein encoded by the gene *SLC4A1*, known as “band 3 protein”, implicated in *P. falciparum* malaria resistance^[52]. Glycophorins are sialoglycoproteins essential for host cell invasion. Their genetic deficiency makes erythrocytes resistant to invasion by *P. falciparum*^[51]. Innate host resistance towards *P. vivax* is expressed by individuals who possess the Duffy blood factor on the surface of RBCs. The Duffy factor is an antigen by which the merozoites of *P. vivax* enter RBCs. Most of sub-Saharan Africans lack this antigenic factor, therefore *P. vivax* is essentially absent from most of this area. Among other innate factors associated with decreased susceptibility to severe malaria, deficiency in glucose-6-phosphate dehydrogenase is of great importance. This enzyme is responsible for preventing the formation of reactive oxygen species from heme groups, thereby slowing down parasite growth^[53,54]. On the contrary, a deficit of haptoglobin (a hemoglobin-binding protein present in plasma which protects tissue from oxidative stress) is associated with an increased susceptibility to severe malaria in specific areas^[55,56]. The increased cytoadherence of *P. falciparum*-infected erythrocytes in small vessels plays a critical role in the pathogenesis of severe malaria. Several endothelial cell adhesion molecules are implicated in this event, binding mainly PfEMP1. These include fatty acid translocase, intracellular adhesion molecule 1, platelet endothelial cell adhesion molecule 1^[57-59]. For these host receptors for cytoadherence, a genetic polymorphism in the promoter region appears to be correlated with the frequency of severe disease^[60-62]. CR1 is considered the most important surface protein that allows infected RBCs to form rosettes and its deficiency is also associated with a high level of genetic polymorphism^[63].

As previously stated, interaction between malaria and the immune system is even more complex when immunological responses are directly involved. A large array of proteins responsible for antigen recognition, antibody response, inflammatory mediation, as well as other serum factors undergo genetic polymorphism in naturally exposed populations. The consequences of this immune gene polymorphism are not always predictable and may vary substantially in different malarious regions due to many others biological reasons^[43]. This issue is the main hurdle to the development of an effective malaria vaccine and the main reason why the use of genotype information for improved malaria treatments and prevention remains a challenge for the future.

Parasite

Of the five *P.* species affecting humans, *P. falciparum* is the most virulent for reasons which are incompletely understood. Some genetic studies indicate that it is the

most recently evolved species, whereas other studies reveal a high level of genetic variation, suggesting a large population size that has been maintained for several hundred thousand years^[64].

P. vivax was the most globally widespread and most prevalent species, until the middle of the 1900s. Currently, it still has the most wide geographic distribution and constitutes the second most common cause of malaria globally with 90% of those infections occurring outside of Africa. This is partly because of its ability to complete the sporogonic cycle at a minimum lower temperature of 16 °C, compared to 21 °C for *P. falciparum*, but (as mentioned above) mainly due to the Duffy polymorphism in Africans which results in a phenotype that does not allow *P. vivax* to invade RBCs. Although *P. vivax*, which can cause relapses months after an infection due to the presence of liver hypnozoites, accounts for 65% of malaria cases in Central and South America and Asia^[65], it causes substantial morbidity with debilitating symptoms but it is rarely fatal. Generally, patients infected with *P. vivax* die as a consequence of splenomegaly. In relation to pregnancy, *P. vivax* (unlike *P. falciparum*) is not associated with shorter gestation or with an increased rate of miscarriage or stillbirth, is instead associated with an increased risk of low birth weight^[66].

P. falciparum and *P. vivax*, which represent the two principal human malaria parasites, seem to be very different in origin and in phylogenetic relationship to other *P.* species^[67]. *P. malariae* is the causative agent of quartan fever. It occurs in various tropical regions throughout the world and causes low, yet significant, morbidity and mortality levels in humans which are associated with renal complications (quartan malarial nephropathy, more common in adults than children)^[68]. Patients may remain parasitemic and asymptomatic for as much as 50 years or more, even in the absence of reinfection. They usually have relapses at irregular intervals, which are actually recrudescences due to the subpatent parasitemia^[69]. The reason of the long duration of *P. malariae* infection may be explained by the slow rate of erythrocytic schizogony of the parasite, and by the inefficacy of common anti-malarial drugs with blood schizonticidal action in eradicating it. Another reason for such a long duration of infection arises because *P. malariae* is the species that has been infecting humans for the longest time. As a consequence, this species has adjusted to the human immune system, which is unable to completely eliminate the parasite.

P. ovale, also a relapsing species, is a rare (0.5%) cause of infection. It is found principally in sub-Saharan Africa and some islands of the western Pacific^[70]. *P. ovale* infections generally follow a benign course, though rare complications may arise due to spleen rupture^[69]. This species was the last of the malaria parasites of humans to be correctly described. It was considered for long time a variant form of *P. vivax* due to the fact that both were found in enlarged and stippled infected erythrocytes. In 1922, Stephens named the parasite *P. ovale* in recognition

of the oval shape of the infected erythrocytes, particularly those containing younger stages of the parasites, which may also show “spiking” or fimbriation^[70]. However, ovalization and fimbriation do not occur spontaneously and cannot be detected by examination of infected blood samples: these transformations are artificially induced at the time of the smear and constitute the best diagnostic tool for species identification^[69].

The fifth *P.* species affecting humans is *P. knowlesi*, an intracellular malaria parasite whose natural vertebrate host is *Macaca fascicularis* (the ‘kra’ monkey). It is now increasingly recognized as a significant cause of malaria in humans, particularly in South East Asia^[71,72]. From a phylogenetic point of view, *P. knowlesi* and *P. vivax* are closely related^[73], although there are important phenotypic differences between them, such as their host blood cell preference, the absence of a dormant liver stage or ‘hypnozoite’ in *P. knowlesi*, and length of the asexual cycle. Recently the *P. knowlesi* [H strain, Pk1(A+) clone] nuclear genome sequence has been described^[74], and it provides an opportunity for comparison with the *P. vivax* genome^[73] and other sequenced *P.* genomes^[75-77].

Evidence is accruing that malaria parasites exhibit a wide range of inter-strain variation within species, patients, and localities, probably because they are exposed to strong selection from the human immune response and treatment with antimalarial drugs. Such a vast strain-specific diversity has a strong impact on clinical manifestations in various age groups and on malaria transmission^[78,79]. However, detecting and understanding parasite maintenance in populations is complicated due to the fact that different evolutionary forces, such as copy number polymorphism and transcriptional variation, help in maintaining the genetic diversity in the parasite genome^[80,81]. It is now quite clear that both *P. falciparum* and *P. vivax* use the genetic diversity they possess to fight against the antimalarial drugs and host immunity although the mechanism of maintenance of such diversity is unknown^[67]. In cases of *P. falciparum* malaria, people living in endemic countries develop only partial immunity, and this immunity correlates with acquisition of strain-specific antibodies that recognize PfEMP1 proteins^[33,82]. The host immune system counters the obstruction of the microcirculation by producing antibodies that interfere with the adhesion of infected RBCs and increase their detectability. The genome of the 3D7 strain of *P. falciparum* contains 59 genes for PfEMP1 proteins^[76]. One gene is expressed at a time, and the parasite avoids detection by varying which PfEMP1 is produced^[83,84]. This ability to switch among a large array of different adhesion ligands is almost certainly the reason why it takes children several years, and many disease episodes, to acquire substantial protective immunity to *P. falciparum* malaria^[21]. Vaccination represents the most direct way to achieve host immunity prior to pathogen exposure. Unfortunately, the malarial parasite constantly changes its immune makeup in every stage of its life cycle, thereby frustrating efforts to produce an effective vaccine.

The development of *P. falciparum* resistance to the most commonly used anti-malarial drugs has been a major cause of failure of any malaria control program and of increasing malarial burden^[85]. Generally, resistance depends on the chemical class of the antimalarial and its mode of action^[86]. Resistance to 4-aminoquinolines, cinchona alkaloids and highly hydrophobic arylmiminoalcohols, arises from mutations of genes encoding vacuolar trans-membrane proteins which regulate the influx/efflux of the drug at the target^[87], whereas there are no well-documented reports on development of resistance against 8-aminoquinolines. Chloroquine (CQ) resistance in *P. falciparum* is primarily attributable to single nucleotide polymorphisms in *pfprt* (CQ resistance transporter)^[88]. Mutations in *P. falciparum* multidrug resistance 1 (PfMDR1), the gene encoding the *P. falciparum* P-glycoprotein homologue-1, seem to be the main cause of resistance to mefloquine but are also implicated in CQ resistance^[89,90]. Resistance to antifolates is quite common worldwide and apparently depends on a stepwise accumulation of single point mutations of genes *pfdhfr* and *pfpr* encoding the drug targets, dihydropteroate synthase and dihydrofolate reductase, respectively^[91]. Atovaquone resistance is associated with single point mutations in the cytochrome *b* gene of *P. falciparum*^[92]. Although artemisinin derivatives represent the most efficacious class of antimalarial drugs, some cases of resistance have been recently detected. This may be due to mutations or amplifications of the gene encoding a PfMDR1 or mutations in the gene encoding sarco-endoplasmic reticulum calcium ATPase^[93,94]. Resistance to one chemical class of antimalarial drugs may cross-react with the others and this is the main reason for the poor efficacy of multi-target antimalarial chemotherapy. This capacity of the malaria parasite to counter the multi-target therapy arises from the fact that gene mutations usually do not act in isolation and act synergistically to encode or enhance resistance. Both mutations in different genes and sequential accumulation of mutations in a single gene may determine cross-resistance^[95].

Mosquito

All human malaria is transmitted through bites by female mosquitoes of genus *Anopheles* (*An.*), but not all anophelines can be considered vectors of malaria. To become a vector, a mosquito has to be susceptible to malaria sporogony, be anthropophilic and have enough longevity to become infective to humans. Mosquito longevity is particularly important because parasite sporogony takes place inside the mosquito midgut over a time span that can vary from 8 d to 30 d, depending on ambient temperature. Usually, sporogony within the mosquito does not occur at temperatures below 16-18 °C. The tropical regions provide ideal living and breeding conditions for the *An.* mosquitoes since temperature, rainfall and humidity are important factors for their survival and distribution. Specific breeding sites in rural areas are bodies of fresh water that are usually large, open,

sunlit and more or less permanent, e.g. swamps (near the edges if deep), weedy sides of streams and channels, rivers, ponds, tanks, wells, furrows or ditches, protected portions of lake shore, rice fields, or water seepages, which are fed from underground permanent sources. In urban and peri-urban areas the preferred breeding sites are building-constructions sites, wells, garden ponds, cisterns, overhead tanks, ground level cement tanks, water coolers^[96-98]. Altitude is another limiting factor for the development of anopheline mosquitoes as, with the exception of a few species, they are generally unable to infect at above 2000 m^[99].

Some *An.* species may be affected by environmental drivers and display behavioural variation (also known as behavioural resistance) within the same species. The latter may determine their occurrence, vectorial status and adaptation to a changing environment. This been recently assessed for *An. sudaicus*, an important malaria vector in coastal areas in Southeast Asian region which can has adapt to breed in a wide range of salinity conditions from fresh water to brackish water^[100]. Behavioural variation has also been observed in the *An. dirus* complex, which includes efficient malaria vectors of the Asian forested zone. Although forest seems essential for survival of this species, adaptations to orchards and wells have been recorded^[101].

Information on the vectors and their precise role in malaria transmission is unclear as all the major malaria vectors are species complexes, which are morphologically indistinguishable but differ significantly in biological characteristics that are vital for malaria control. These include vectorial potential, host-preference, distribution pattern, resting behavior and response to insecticides. These sibling (or cryptic) species can be recognized only through chromosomal studies or biochemical analysis^[102]. For instance, the *An. culicifacies* complex, which constitutes the main vector system in Indian with an overall rate of 60%-65% of malaria cases^[103], comprises five sibling species provisionally designated as species A, B, C, D and E. Among them, species B holds special features: (1) it is the only member of *culicifacies* complex regarded as a non-vector; (2) it is found in isolation in eastern India, while in other areas of Indian sub-continent it is found in association with species C, D or E^[104]; (3) it has the ability to kill malaria parasites in the midgut during early sporogony by a process of encapsulation^[105]; and (4) it develops resistance towards insecticides [dichlorodiphenyltrichloroethane (DDT) and malathion] at a faster rate than species A and/or sympatric species B^[106-108]. *An. anthropophagus* and *An. sinensis* constitute another example of cryptic species which are morphologically indistinguishable. They are the main vectors in central China where they mostly occur in symmetry. Identification of their genetic diversity could facilitate studies on malaria transmission and the development of prevention strategies for malaria control^[109].

Although there are 444 formally named species and 40 unnamed members of species complexes recognized

as distinct morphological and/or genetic *An.* species^[110], only 60 of them transmit malaria under natural conditions, and only 30 are of major importance^[111]. Of these, the *An. gambiae* complex and *An. funestus* are the most efficient vectors of *P. falciparum* malaria in Sub-Saharan Africa^[112]. *An. gambiae* exhibits the highest rates of sporozoite development, thus it represents one of the prime targets for genetic modification projects^[113]. This species complex consists of six sibling species (*An. arabiensis*, *An. bwambae*, *An. merus*, *An. melas*, *An. quadriannulatus*, *An. gambiae* sensu stricto) with different behavioural traits^[114]. Within this species complex, *An. gambiae* s. str. and *An. arabiensis* are the major vectors of human malaria in sub-Saharan Africa, with *An. merus* and *An. melas* being intermediate in importance^[115]. This last two species are associated with salt-water with a localized distribution along the eastern and western coasts of Africa, respectively^[116,117]. *An. bwambae* and *An. quadriannulatus* (species A, found in south-east Africa, and species B, which has been described in Ethiopia) are highly zoophilic and are never or rarely exposed to the human *P. falciparum*, thus they are not considered vectors of human malaria^[115,118]. *An. bwambae* (which has only been found breeding in mineral springs) has the most restricted range, limited to the Semliki Forest of Uganda^[119]. *An. gambiae* s. str. is currently in a state of diverging into different species - the Mopti (M) and Savannah (S) strains - though as of 2007, the two strains were still considered to be a single species. The *An. gambiae* s. str. genome has been sequenced by Holt *et al*^[120] and revised by Sharakhova *et al*^[121], though there is controversy over the choice of strain used, which is considered a hybrid of two different strains.

In the Pacific area *An. farauti* complex, *An. maculatus* and *An. flavirostris* play a predominant role in malaria transmission. These species are geographically isolated, with the latter confined mainly to the Philippines, much of Indonesia, and Sabah, Malaysia^[122-124]. Actually, *An. flavirostris* is considered a subspecies of *An. minimus*, a complex that counts five sibling species and represents one of the most important vectors of human malaria in East Asian countries (Nepal, Bangladesh, north Thailand, Indonesia, south China and also in the Yaeyama Island of Japan)^[125]. The *An. farauti* complex belongs to the *An. punctulatus* group, which includes at least 12 sibling species and is widespread in northern Australia (wherein it is not a malaria vector) and the islands of the south-west Pacific^[126]. Four of the twelve species have been implicated as malaria vectors in Papa New Guinea^[127]. *An. albimanus* is the primary coastal vector in South America, Central America and the Caribbean^[128]. *An. aquasalis* is a coastal Neotropical species, considered to be the primary coastal malaria vector of *P. vivax* in Venezuela^[129]. Another important malaria vector with a broad geographical distribution ranging from southern Mexico to northern Argentina is *An. darlingi*. Its degree of involvement in human malaria transmission seems to differ among localities^[130]. Besides its morphological, behavioral and genetic diversity, *An. darlingi* spread

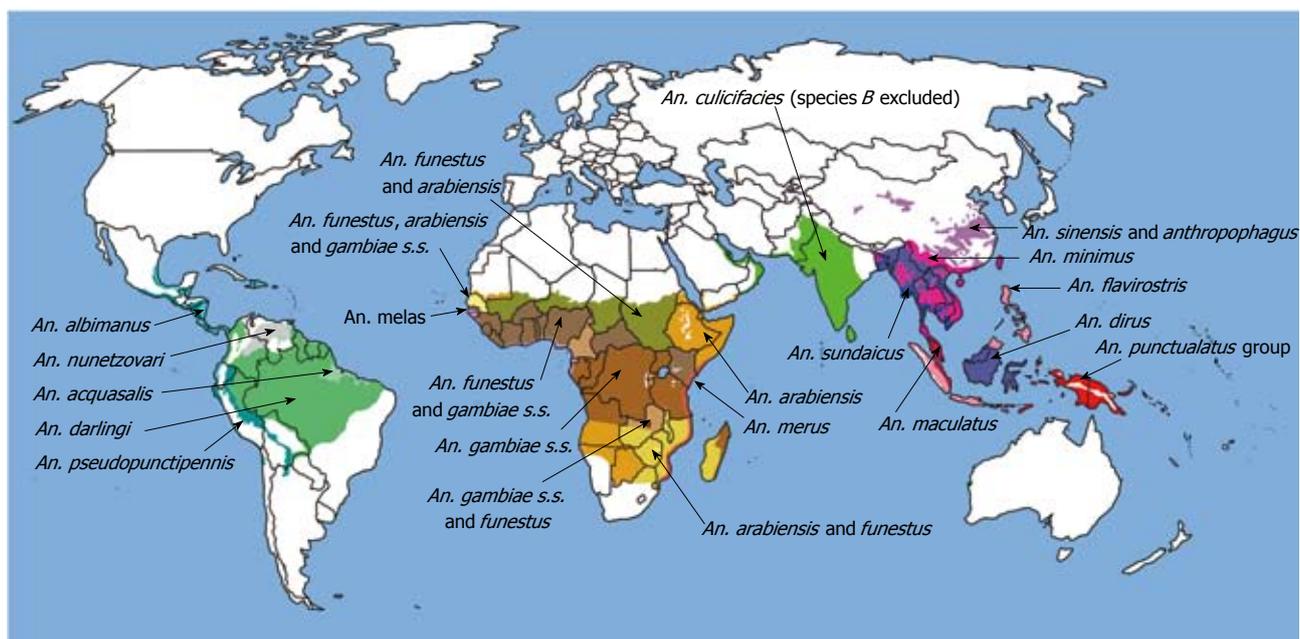


Figure 2 Map of the world showing the distribution of the most important anthropophilic *Anopheles* mosquitoes which are currently considered vectors of malaria. An.: *Anopheles*; s.s.: *sensu stricto*

throughout the Amazon basin (where it is considered the main vector) has been recently associated with the uncontrolled rate of deforestation^[131]. *An. nuneztovari* is considered an important vector of human malaria in areas of Venezuela and Colombia, although populations of this species occupy large geographic areas (northern South America, eastern Panama, Brazil and Amazon region states)^[132]. Among the dominant *Anopheles* vectors of human malaria in the Americas it is worth noting the *An. pseudopunctipennis* complex since it can survive and transmit malaria at altitudes up to 3000 m^[133]. The geographic distribution of the mentioned human malaria vectors is depicted in Figure 2.

Vector control has had limited success in areas where malaria is heavily endemic, primarily due to the lack of resources for disease management. The options available for vector control are mainly, insecticides, personal protection measures, larval control, biological control and environmental management. Pyrethroids are the only insecticides that have been used for impregnation of bed-nets because of their very low mammalian toxicity. Trials of long-lasting insecticide-treated nets impregnated with permethrin, deltamethrin and alphacypermethrin are underway^[134]. Unfortunately, there is an increasing resistance in vectors towards pyrethroids and a well established resistance towards other older insecticides used as indoor residual sprays or in public health. Mosquitoes exhibit a broad armamentarium for physiological defense. Their major mechanisms of resistance include: glutathione-S-transferase-based degradation of DDT, carboxyl esterase-dependent hydrolysis of malathion, altered acetyl cholinesterase activity to organophosphates and carbamates, cytochrome P-450 monooxygenase and *kdr* type resistance against pyrethroids^[135]. Larval control may represent an alternative strategy for mosquito abate-

ment, but it is a laborious challenge that requires practical environmental and entomological skills at all levels, and thus is only achievable in urban and peri-urban situations. Environmental control is used to prevent breeding, nesting, and feeding of vectors and, as for larval control, requires community participation and intersectoral collaboration. All these prevention measures will have a paltry impact on transmission and a limited success in decreasing the malaria burden when applied alone. Thus, newer and more advanced vector-focused approaches are needed^[136].

PLASMODIUM METABOLISM AND POSSIBLE DRUG TARGETS

The malaria parasite possesses unique mechanisms for the synthesis of biomolecules and a better understanding of its metabolic pathways may lead to the development of novel therapeutic strategies.

Hemoglobin degradation pathway

The malaria parasite grows rapidly during many stages of its life cycle within the human host and exhibits a high multiplication rate. This necessitates a constant source of nutrients, both for protein biosynthesis and energy metabolism, which mainly come from ingested hemoglobin during the morphologically separate phases inside the erythrocyte (ring stage, trophozoite stage, and schizont stage)^[137,138]. The parasite degrades a large amount of host cell hemoglobin by means of a variety of proteases which are thought to act in semi-ordered fashion^[139], and whose importance as a drug targets is well-documented^[140,141]. This massive digestive process (up to 65% of the total host hemoglobin) occurs in a specialized organelle, the

food vacuole, which requires a pH optimum in the range 4.5-5.0^[142].

Two closely related aspartic proteases, termed plasmeprin (PM) I and II, are involved in the early events of hemoglobin degradation, promoting unfolding of globin chains and release of the heme moiety. Further digestion of globin chains is carried out by at least other two aspartic proteases, PM IV and histo-aspartic proteinase^[140,143], and three cysteine proteases termed falcipains (FPs)^[141,144-146]. The cysteine proteases involved in the hemoglobin catabolism are two nearly identical copies of FP-2 (FP-2 and FP-2', also known as FP-2A and FP-2B respectively)^[146,147], and FP-3^[144]. Both PMs and FPs are synthesized as membrane-bound proforms that are transported to the food vacuole and activated by means of overlapping, redundant mechanisms^[148].

A zinc metalloprotease (named falcilysin) intervenes later in this digestive process since it is able to cleave only small polypeptides (up to 20 amino acids)^[149] that are eventually shortened further by a dipeptidyl aminopeptidase 1^[150]. Oligopeptides are then pumped out of the food vacuole and an amino peptidase activity within the parasite cytoplasm provides amino acids essential for parasites survival^[151,152]. Despite this substantial proteolysis, malaria parasites employ only 16% of the digested hemoglobin for biosynthesis of proteins^[153]. Most of newly obtained amino acids are effluxed from the infected erythrocyte to provide space for the growing parasite and maintain osmotic stability^[154].

Massive degradation of hemoglobin also leads to generation of a large quantity of heme that is toxic to the parasite, promoting membrane damage due to its oxidative properties^[155]. Released heme is detoxified to a cyclic dimer, β -hematin. The low digestive vacuolar pH promotes crystallization and polymerization of these dimers to give hemozoin pigment^[156,157]. This defense mechanism from oxidative stress is most pronounced during the trophozoite stage of the parasite development^[158]. More recent studies indicate that the crystallization process from β -hematin dimers to hemozoin takes place in, or closely associated with, neutral lipid nanospheres in the aqueous content of the vacuole^[159]. Despite accumulating heme derived from RBC hemoglobin, malarial parasites synthesize heme *de novo* for metabolic use. The heme biosynthetic pathway of the parasite is similar to that of humans and animals, using glycine and succinyl-CoA to make δ -aminolevulinic acid (ALA), the committed precursor for heme biosynthesis. However, while the parasite makes its own ALA, it has to import ALA dehydratase and ferrochelatase from host RBC to complete the pathway^[160,161].

Purine pathway

Plasmodium does not manifest the *de novo* pathway for purine nucleotide biosynthesis and depends on the salvage pathway for its supply of purine^[162]. Purine uptake into the intraerythrocytic malaria parasite involves four different nucleobase/nucleoside transporters localized to the

parasite plasma membrane, two with a high substrate affinity and two with a low substrate affinity^[163-166]. The first comprehensive model for purine uptake by *P. falciparum* has been proposed by Quashie *et al*^[167]. Their studies report on the presence of a low affinity adenosine transporter, a high affinity adenine transporter, a low affinity uptake route for adenine and a high affinity hypoxanthine/purine nucleoside transporter (PfNT1, also known as PfENT1). Recently, the role of PfNT1 in the purine salvage pathway has been called into question by other authors who assert that the high-affinity uptake components characterized by Quashie *et al*^[167] are strictly related to the high-affinity intracellular metabolism (and perhaps sequestration) of purines carried out by *Plasmodium* rather than the presence of high-affinity transporters, reaffirming a low-affinity system for PfNT1^[168,169]. Studies on PfNT1 knock-out parasites have also indicated, one or more additional transport pathways (of unknown affinity) for the uptake of adenine^[170]. Hypoxanthine is regarded as the key metabolic precursor of all other purines. The source of hypoxanthine is believed to be the adenosine triphosphate present in the infected erythrocytes, which is catabolized to hypoxanthine *via* adenosine diphosphate, adenosine monophosphate, adenosine and inosine^[171]. In *Plasmodium*, adenosine is converted to inosine by adenosine deaminase^[172], which is further metabolized to hypoxanthine by purine nucleoside phosphorylase^[173].

Pyrimidine and co-factors of the B-complex vitamin pathway

Unlike purine biosynthesis, the malaria parasite lacks the salvage pathway for pyrimidines and synthesizes them *de novo*^[174]. To accomplish this, the parasite usually synthesizes itself the required folate co-factors (vitamin B₉), starting with the condensation of pteridine with para-aminobenzoic acid to form dihydropteroic acid. The latter is then condensed with glutamic acid to dihydrofolic acid and lastly reduced to tetrahydrofolic acid, which is the active form of vitamin B₉ essential in one-carbon transfer reactions^[175]. Humans only have the ability to convert folic acid into tetrahydrofolic acid and this is the basis of the selective toxicity of type-I antifolates (dihydropteroate synthase inhibitors) used occasionally as antimalarial drugs in association with type-II antifolates (dihydrofolate reductase inhibitors)^[176]. Among the six enzymes involved in pyrimidine biosynthesis, dihydroorotate dehydrogenase (the fourth one) is the most exploited as a drug target^[177].

In addition to vitamin B₉, the malaria parasite is able to achieve *de novo* synthesis of other important co-factors of B complex (B₁ and B₆) which do not occur in humans and therefore represent potentially powerful drug targets^[178]. Biosynthesis of thiamine pyrophosphate (the active form of vitamin B₁) takes place *via* two branches, the thiazole and pyrimidine pathways, which join to form the key intermediate thiamine monophosphate. Two different distinct pathways, generate pyridoxal 5-phosphate

(the active form of vitamin B₆). One is related to the pentose phosphate shunt and glycolysis, while the other depends on the salvage of B₆ vitamins^[179]. Both vitamins B₁ and B₆ are transported primarily in erythrocytes, raising the question of why *Plasmodium* uses energy to produce its own co-factors. For vitamin B₆, the reason resides in the fact that it is tightly bound to serum albumin and hemoglobin and thus might not be available to the parasite^[179]. However, it has been demonstrated that the *de novo* synthesis of vitamins B₁ and B₆ by the parasite is not sufficient for its survival during erythrocytic schizogony^[180-182]. Vitamin B₁ is an essential co-factor of enzyme complexes such as pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, branched-chain 2-oxo acid dehydrogenase and transketolase, thus involving diverse and connected metabolic pathways. Vitamin B₆ acts as a carbonyl-reactive co-factor for more than 140 distinct enzymatic reactions, all involved in the conversion of amino acids *via* processes such as decarboxylation and transamination^[179].

Lipid pathway

P. species, like other apicomplexan parasites, have a special type II fatty acid biosynthetic pathway (FAS II) whose enzymes are localized in the apicoplast^[183]. This organelle is a plastid of cyanobacterial origin and it is closely associated with the mitochondrion at all stages of parasite development^[184]. The apicoplast-mitochondrion association is also involved in other biosynthetic processes such as isoprenoid and ubiquinone biosynthesis^[174]. *Plasmodium* FAS II consists of a chain elongation pathway which depends on four key enzymes (in sequence: FabB/E, FabG, FabZ and FabI) and leads to the formation of fatty acids with between 10 and 14 carbons linked to an acyl carrier protein^[185]. All the four key enzymes of *Plasmodium* FAS II represent promising drug targets because of their bacterial origin^[186]. Although as a rule the malaria parasite scavenges fatty acids from its host, it has been recently demonstrated that FAS II is essential for its liver-stage development^[187].

During the erythrocyte stage, the rapid growth of the parasite is fueled by other precursors such as serine, ethanolamine, and choline which represent the major building blocks for the *de novo* synthesis of structural and regulatory phospholipids. The enzymes involved in these biosynthetic pathways are either absent from humans, or markedly different from their human counterparts, and thus represent other important drug targets^[186].

Glucose pathway

In the *P. falciparum* the mitochondrion is atypical. During the asexual stage, it lacks cristae, but possibly develops cristae in the gametocyte stage^[188]. The role of the parasite mitochondrion has not yet been completely clarified, though the main functions of a typical mitochondrion have been assessed. It is involved in energy generation through glycolysis, electron transport *via* flavin adenine dinucleotide-linked tricarboxylic acid cycle enzymes,

and intermediary metabolism^[189]. Glucose is the primary source of energy but the metabolic steps involved in the conversion of glucose to lactate (approximately 85%) are essentially the same as that found in other organisms. Therefore, the glycolytic pathway of *Plasmodium* has not been investigated as a potential drug target as much as other pathways. In this context, a parasite-derived carrier protein called *P. falciparum* hexose transporter is more important as a drug target due to its superior affinity for glucose as compared to human Glucose transporter 1^[190].

Other pathways under evaluation

There are several other secondary metabolic pathways of the malaria parasite that are currently under consideration as valid drug targets^[191].

Antioxidant enzymes in the redox pathway, such as glutathione reductase, glutathione S-transferase, and thioredoxin reductase can be exploited as antimalarial drug targets since they differ sufficiently from the host enzymes.

Staines *et al*^[192] have reported the induction by the parasite of new permeability pathways towards the membrane of infected RBCs. These pathways are mainly anion specific channels that also increase the influx of cations and small electroneutral solutes (although at a lower rate).

Plasmodial cyclin-dependent kinases are highly conserved and play a pivotal role in parasite growth and development, and are currently being exploited for the development of selective inhibitors^[193].

The shikimate pathway is absent in human and all other mammals, but it is essential for parasite growth. Thus, it is being thoroughly investigated for the development of new antimalarial agents^[194]. This pathway is an important metabolic route that links carbohydrate metabolism to the biosynthesis of aromatic compounds such as aminoacids and precursors of other essential co-factors (ubiquinone and folic acid).

Aquaporins are integral membrane proteins that regulate the flow of water or uncharged polar solutes. They protect the parasite from the osmotic stress encountered during its development inside the erythrocyte as well as during its passage through the kidney of the human host. Moreover, they help the parasite to acquire nutrients from the host organism and to eliminate metabolites. The structure of plasmodial aquaporins differs from that of human aquaporins at the level of the accessible pore mouth. Therefore, aquaporins represent a valid alternative target for the development of new antimalarial agents^[195].

CONCLUSION

In spite of the significant recent advances in antimicrobial chemotherapy, supportive care and vector control strategies, malaria still represents a serious threat to human health, killing about one million people per year worldwide. Part of the inability to combat this disease is attributed to an incomplete understanding of its patho-

genesis and pathophysiology. Improving the knowledge of the underlying pathogenic mechanisms of malaria transmission and of the exclusive metabolic pathways carried out by the parasite, should promote the efficiency of the treatment of disease and help the identification of novel targets for potential therapeutic interventions. Moreover, the elucidation of determinants involved in the spread of malaria - which we have hopefully sufficiently covered within this review - will provide important information for efficient planning of strategies for targeted control.

REFERENCES

- World Malaria Report 2011. Available from: URL: http://www.who.int/malaria/world_malaria_report_2011/en
- Reis BA, Mashkov OA, Karmanov PA, Toguzov RT. [Toxin study in peritonitis]. *Khirurgiia* (Mosk) 1983; (6): 77-80
- Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, Thomas A, Conway DJ. A large focus of naturally acquired Plasmodium knowlesi infections in human beings. *Lancet* 2004; **363**: 1017-1024
- Gething PW, Patil AP, Smith DL, Guerra CA, Elyazar IR, Johnston GL, Tatem AJ, Hay SI. A new world malaria map: Plasmodium falciparum endemicity in 2010. *Malar J* 2011; **10**: 378
- Ogbonna A, Uneke CJ. Artemisinin-based combination therapy for uncomplicated malaria in sub-Saharan Africa: the efficacy, safety, resistance and policy implementation since Abuja 2000. *Trans R Soc Trop Med Hyg* 2008; **102**: 621-627
- Schlitzer M. Antimalarial drugs - what is in use and what is in the pipeline. *Arch Pharm* (Weinheim) 2008; **341**: 149-163
- Na-Bangchang K, Karbwang J. Current status of malaria chemotherapy and the role of pharmacology in antimalarial drug research and development. *Fundam Clin Pharmacol* 2009; **23**: 387-409
- Trampuz A, Jereb M, Muzlovic I, Prabhu RM. Clinical review: Severe malaria. *Crit Care* 2003; **7**: 315-323
- Brown GV, Beck HP, Molyneux M, Marsh K. Molecular approaches to epidemiology and clinical aspects of malaria. *Parasitol Today* 2000; **16**: 448-451
- Khan SM, Waters AP. Malaria parasite transmission stages: an update. *Trends Parasitol* 2004; **20**: 575-580
- Iyer J, Grüner AC, Rénia L, Snounou G, Preiser PR. Invasion of host cells by malaria parasites: a tale of two protein families. *Mol Microbiol* 2007; **65**: 231-249
- Wegscheid-Gerlach C, Gerber HD, Diederich WE. Proteases of Plasmodium falciparum as potential drug targets and inhibitors thereof. *Curr Top Med Chem* 2010; **10**: 346-367
- Sarkar S, Bhattacharya P. Cerebral malaria caused by Plasmodium vivax in adult subjects. *Indian J Crit Care Med* 2008; **12**: 204-205
- Mackintosh CL, Beeson JG, Marsh K. Clinical features and pathogenesis of severe malaria. *Trends Parasitol* 2004; **20**: 597-603
- Horrocks P, Pinches RA, Chakravorty SJ, Papakrivov J, Christodoulou Z, Kyes SA, Urban BC, Ferguson DJ, Newbold CI. PfEMP1 expression is reduced on the surface of knobless Plasmodium falciparum infected erythrocytes. *J Cell Sci* 2005; **118**: 2507-2518
- Anstey NM, Weinberg JB, Hassanali MY, Mwaikambo ED, Manyenga D, Misukonis MA, Arnelle DR, Hollis D, McDonald MI, Granger DL. Nitric oxide in Tanzanian children with malaria: inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. *J Exp Med* 1996; **184**: 557-567
- Anstey NM, Granger DL, Hassanali MY, Mwaikambo ED, Duffy PE, Weinberg JB. Nitric oxide, malaria, and anemia: inverse relationship between nitric oxide production and hemoglobin concentration in asymptomatic, malaria-exposed children. *Am J Trop Med Hyg* 1999; **61**: 249-252
- Obi RK, Okangba CC, Nwanebu FC, Ndubuisi UU, Orji NM. Premunition in Plasmodium falciparum malaria. *Afr J Biotechnol* 2010; **9**: 1397-1401
- Francis SE, Sullivan DJ, Goldberg DE. Hemoglobin metabolism in the malaria parasite Plasmodium falciparum. *Annu Rev Microbiol* 1997; **51**: 97-123
- Gowda DC. Role of chondroitin-4-sulfate in pregnancy-associated malaria. *Adv Pharmacol* 2006; **53**: 375-400
- Hviid L. Adhesion specificities of Plasmodium falciparum-infected erythrocytes involved in the pathogenesis of pregnancy-associated malaria. *Am J Pathol* 2007; **170**: 1817-1819
- Muthusamy A, Achur RN, Bhavanandan VP, Fouda GG, Taylor DW, Gowda DC. Plasmodium falciparum-infected erythrocytes adhere both in the intervillous space and on the villous surface of human placenta by binding to the low-sulfated chondroitin sulfate proteoglycan receptor. *Am J Pathol* 2004; **164**: 2013-2025
- Muthusamy A, Achur RN, Valiyaveetil M, Botti JJ, Taylor DW, Leke RF, Gowda DC. Chondroitin sulfate proteoglycan but not hyaluronic acid is the receptor for the adherence of Plasmodium falciparum-infected erythrocytes in human placenta, and infected red blood cell adherence up-regulates the receptor expression. *Am J Pathol* 2007; **170**: 1989-2000
- Alkhalil A, Achur RN, Valiyaveetil M, Ockenhouse CF, Gowda DC. Structural requirements for the adherence of Plasmodium falciparum-infected erythrocytes to chondroitin sulfate proteoglycans of human placenta. *J Biol Chem* 2000; **275**: 40357-40364
- Fried M, Lauder RM, Duffy PE. Plasmodium falciparum: adhesion of placental isolates modulated by the sulfation characteristics of the glycosaminoglycan receptor. *Exp Parasitol* 2000; **95**: 75-78
- Chai W, Beeson JG, Lawson AM. The structural motif in chondroitin sulfate for adhesion of Plasmodium falciparum-infected erythrocytes comprises disaccharide units of 4-O-sulfated and non-sulfated N-acetylgalactosamine linked to glucuronic acid. *J Biol Chem* 2002; **277**: 22438-22446
- Achur RN, Valiyaveetil M, Gowda DC. The low sulfated chondroitin sulfate proteoglycans of human placenta have sulfate group-clustered domains that can efficiently bind Plasmodium falciparum-infected erythrocytes. *J Biol Chem* 2003; **278**: 11705-11713
- Sugitan AL, Leke RG, Fouda G, Zhou A, Thuita L, Metenou S, Fogako J, Megnekou R, Taylor DW. Changes in the levels of chemokines and cytokines in the placentas of women with Plasmodium falciparum malaria. *J Infect Dis* 2003; **188**: 1074-1082
- McGregor IA, Wilson ME, Billewicz WZ. Malaria infection of the placenta in The Gambia, West Africa; its incidence and relationship to stillbirth, birthweight and placental weight. *Trans R Soc Trop Med Hyg* 1983; **77**: 232-244
- Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, Kahigwa E, Font F, Alonso PL. The impact of placental malaria on gestational age and birth weight. *J Infect Dis* 2000; **181**: 1740-1745
- Brabin BJ, Romagosa C, Abdelgalil S, Menéndez C, Verhoeff FH, McGready R, Fletcher KA, Owens S, D'Alessandro U, Nosten F, Fischer PR, Ordi J. The sick placenta-the role of malaria. *Placenta* 2004; **25**: 359-378
- Beeson JG, Duffy PE. The immunology and pathogenesis of malaria during pregnancy. *Curr Top Microbiol Immunol* 2005; **297**: 187-227
- Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE. Maternal antibodies block malaria. *Nature* 1998; **395**: 851-852
- Dzikowski R, Templeton TJ, Deitsch K. Variant antigen gene expression in malaria. *Cell Microbiol* 2006; **8**: 1371-1381

- 35 **Molineaux L.** Malaria and mortality: some epidemiological considerations. *Ann Trop Med Parasitol* 1997; **91**: 811-825
- 36 **Rogerson SJ, Wijesinghe RS, Meshnick SR.** Host immunity as a determinant of treatment outcome in *Plasmodium falciparum* malaria. *Lancet Infect Dis* 2010; **10**: 51-59
- 37 **van der Heyde HC, Nolan J, Combes V, Gramaglia I, Grau GE.** A unified hypothesis for the genesis of cerebral malaria: sequestration, inflammation and hemostasis leading to microcirculatory dysfunction. *Trends Parasitol* 2006; **22**: 503-508
- 38 **Wilson NO, Huang MB, Anderson W, Bond V, Powell M, Thompson WE, Armah HB, Adjei AA, Gyasi R, Tettey Y, Stiles JK.** Soluble factors from *Plasmodium falciparum*-infected erythrocytes induce apoptosis in human brain vascular endothelial and neuroglia cells. *Mol Biochem Parasitol* 2008; **162**: 172-176
- 39 **Aidoo M, Terlouw DJ, Kolczak MS, McElroy PD, ter Kuile FO, Kariuki S, Nahlen BL, Lal AA, Udhayakumar V.** Protective effects of the sickle cell gene against malaria morbidity and mortality. *Lancet* 2002; **359**: 1311-1312
- 40 **Ringelhan B, Hathorn MK, Jilly P, Grant F, Parniczky G.** A new look at the protection of hemoglobin AS and AC genotypes against *Plasmodium falciparum* infection: a census tract approach. *Am J Hum Genet* 1976; **28**: 270-279
- 41 **Chotivanich K, Udomsangpetch R, Pattanapanyasat K, Chierakul W, Simpson J, Looareesuwan S, White N.** Hemoglobin E: a balanced polymorphism protective against high parasitemias and thus severe *P falciparum* malaria. *Blood* 2002; **100**: 1172-1176
- 42 **Taylor SM, Parobek CM, Fairhurst RM.** Haemoglobinopathies and the clinical epidemiology of malaria: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; **12**: 457-468
- 43 **Kwiatkowski DP.** How malaria has affected the human genome and what human genetics can teach us about malaria. *Am J Hum Genet* 2005; **77**: 171-192
- 44 **Agarwal A, Guindo A, Cissoko Y, Taylor JG, Coulibaly D, Koné A, Kayentao K, Djimde A, Plowe CV, Doumbo O, Wellems TE, Diallo D.** Hemoglobin C associated with protection from severe malaria in the Dogon of Mali, a West African population with a low prevalence of hemoglobin S. *Blood* 2000; **96**: 2358-2363
- 45 **Modiano D, Luoni G, Sirima BS, Simporé J, Verra F, Konaté A, Rastrelli E, Olivieri A, Calissano C, Paganotti GM, D'Urbano L, Sanou I, Sawadogo A, Modiano G, Coluzzi M.** Haemoglobin C protects against clinical *Plasmodium falciparum* malaria. *Nature* 2001; **414**: 305-308
- 46 **Mockenhaupt FP, Ehrhardt S, Cramer JP, Ochwemah RN, Anemana SD, Goltz K, Mylius F, Dietz E, Egelte TA, Bienzle U.** Hemoglobin C and resistance to severe malaria in Ghanaian children. *J Infect Dis* 2004; **190**: 1006-1009
- 47 **Rihet P, Flori L, Tall F, Traore AS, Fumoux F.** Hemoglobin C is associated with reduced *Plasmodium falciparum* parasitemia and low risk of mild malaria attack. *Hum Mol Genet* 2004; **13**: 1-6
- 48 **Ohashi J, Naka I, Patarapotikul J, Hananantachai H, Brittenham G, Looareesuwan S, Clark AG, Tokunaga K.** Extended linkage disequilibrium surrounding the hemoglobin E variant due to malarial selection. *Am J Hum Genet* 2004; **74**: 1198-1208
- 49 **Mockenhaupt FP, Ehrhardt S, Gellert S, Ochwemah RN, Dietz E, Anemana SD, Bienzle U.** Alpha(+)-thalassemia protects African children from severe malaria. *Blood* 2004; **104**: 2003-2006
- 50 **Genton B, al-Yaman F, Mgone CS, Alexander N, Panu MM, Alpers MP, Mokela D.** Ovalocytosis and cerebral malaria. *Nature* 1995; **378**: 564-565
- 51 **Wang HY, Tang H, Shen CK, Wu CI.** Rapidly evolving genes in human. I. The glycoporphins and their possible role in evading malaria parasites. *Mol Biol Evol* 2003; **20**: 1795-1804
- 52 **Allen SJ, O'Donnell A, Alexander ND, Mgone CS, Peto TE, Clegg JB, Alpers MP, Weatherall DJ.** Prevention of cerebral malaria in children in Papua New Guinea by southeast Asian ovalocytosis band 3. *Am J Trop Med Hyg* 1999; **60**: 1056-1060
- 53 **Roth EF, Raventos-Suarez C, Rinaldi A, Nagel RL.** Glucose-6-phosphate dehydrogenase deficiency inhibits in vitro growth of *Plasmodium falciparum*. *Proc Natl Acad Sci USA* 1983; **80**: 298-299
- 54 **Janney SK, Joist JJ, Fitch CD.** Excess release of ferriheme in G6PD-deficient erythrocytes: possible cause of hemolysis and resistance to malaria. *Blood* 1986; **67**: 331-333
- 55 **Elagib AA, Kider AO, Akerström B, Elbashir MI.** Association of the haptoglobin phenotype (1-1) with *falciparum* malaria in Sudan. *Trans R Soc Trop Med Hyg* 1998; **92**: 309-311
- 56 **Aucan C, Walley AJ, Greenwood BM, Hill AV.** Haptoglobin genotypes are not associated with resistance to severe malaria in The Gambia. *Trans R Soc Trop Med Hyg* 2003; **96**: 327-328
- 57 **Oquendo P, Hundt E, Lawler J, Seed B.** CD36 directly mediates cytoadherence of *Plasmodium falciparum* parasitized erythrocytes. *Cell* 1989; **58**: 95-101
- 58 **Berendt AR, Simmons DL, Tansey J, Newbold CI, Marsh K.** Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. *Nature* 1989; **341**: 57-59
- 59 **Treutiger CJ, Heddi A, Fernandez V, Muller WA, Wahlgren M.** PECAM-1/CD31, an endothelial receptor for binding *Plasmodium falciparum*-infected erythrocytes. *Nat Med* 1997; **3**: 1405-1408
- 60 **Omi K, Ohashi J, Patarapotikul J, Hananantachai H, Naka I, Looareesuwan S, Tokunaga K.** CD36 polymorphism is associated with protection from cerebral malaria. *Am J Hum Genet* 2003; **72**: 364-374
- 61 **Fernandez-Reyes D, Craig AG, Kyes SA, Peshu N, Snow RW, Berendt AR, Marsh K, Newbold CI.** A high frequency African coding polymorphism in the N-terminal domain of ICAM-1 predisposing to cerebral malaria in Kenya. *Hum Mol Genet* 1997; **6**: 1357-1360
- 62 **Kikuchi M, Looareesuwan S, Ubalee R, Tasanor O, Suzuki F, Wattanagoon Y, Na-Bangchang K, Kimura A, Aikawa M, Hirayama K.** Association of adhesion molecule PECAM-1/CD31 polymorphism with susceptibility to cerebral malaria in Thailand. *Parasitol Int* 2001; **50**: 235-239
- 63 **Cockburn IA, Mackinnon MJ, O'Donnell A, Allen SJ, Moulds JM, Baisor M, Bockarie M, Reeder JC, Rowe JA.** A human complement receptor 1 polymorphism that reduces *Plasmodium falciparum* rosetting confers protection against severe malaria. *Proc Natl Acad Sci USA* 2004; **101**: 272-277
- 64 **Hey J.** Parasite populations: the puzzle of *Plasmodium*. *Curr Biol* 1999; **9**: R565-R567
- 65 **Mendis K, Sina BJ, Marchesini P, Carter R.** The neglected burden of *Plasmodium vivax* malaria. *Am J Trop Med Hyg* 2001; **64**: 97-106
- 66 **Nosten F, McGready R, Simpson JA, Thwai KL, Balkan S, Cho T, Hkirijsaroen L, Looareesuwan S, White NJ.** Effects of *Plasmodium vivax* malaria in pregnancy. *Lancet* 1999; **354**: 546-549
- 67 **Das A, Bajaj R, Mohanty S, Swain V.** Genetic diversity and evolutionary history of *Plasmodium falciparum* and *P. vivax*. *Curr Sci* 2007; **92**: 1516-1524
- 68 **Benitez JA, Rodriguez Morales AJ.** Epidemiology of *Plasmodium malariae* infections in Venezuela. *Acta Cient Estud* 2007; **5**: 186-188
- 69 **Swierczynski G, Gobbo M.** Atlas of human malaria. Sirnion, Italy: AzColor, 2007
- 70 **Collins WE, Jeffery GM.** *Plasmodium ovale*: parasite and disease. *Clin Microbiol Rev* 2005; **18**: 570-581
- 71 **Cox-Singh J, Davis TM, Lee KS, Shamsul SS, Matusop A, Ratnam S, Rahman HA, Conway DJ, Singh B.** *Plasmodium knowlesi* malaria in humans is widely distributed and po-

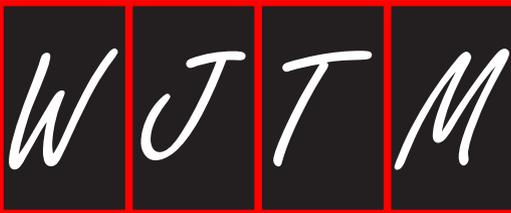
- tentially life threatening. *Clin Infect Dis* 2008; **46**: 165-171
- 72 **White NJ**. Plasmodium knowlesi: the fifth human malaria parasite. *Clin Infect Dis* 2008; **46**: 172-173
- 73 **Carlton JM**, Adams JH, Silva JC, Bidwell SL, Lorenzi H, Caler E, Crabtree J, Angiuoli SV, Merino EF, Amedeo P, Cheng Q, Coulson RM, Crabb BS, Del Portillo HA, Essien K, Feldblyum TV, Fernandez-Becerra C, Gilson PR, Gueye AH, Guo X, Kang'a S, Kooij TW, Korsinczky M, Meyer EV, Nene V, Paulsen I, White O, Ralph SA, Ren Q, Sargeant TJ, Salzberg SL, Stoeckert CJ, Sullivan SA, Yamamoto MM, Hoffman SL, Wortman JR, Gardner MJ, Galinski MR, Barnwell JW, Fraser-Liggett CM. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. *Nature* 2008; **455**: 757-763
- 74 **Pain A**, Böhme U, Berry AE, Mungall K, Finn RD, Jackson AP, Mourier T, Mistry J, Pasini EM, Aslett MA, Balasubramaniam S, Borgwardt K, Brooks K, Carret C, Carver TJ, Cherevach I, Chillingworth T, Clark TG, Galinski MR, Hall N, Harper D, Harris D, Hauser H, Ivens A, Janssen CS, Keane T, Larke N, Lapp S, Marti M, Moule S, Meyer IM, Ormond D, Peters N, Sanders M, Sanders S, Sargeant TJ, Simmonds M, Smith F, Squares R, Thurston S, Tivey AR, Walker D, White B, Zuiderwijk E, Churcher C, Quail MA, Cowman AF, Turner CM, Rajandream MA, Kocken CH, Thomas AW, Newbold CI, Barrell BG, Berriman M. The genome of the simian and human malaria parasite *Plasmodium knowlesi*. *Nature* 2008; **455**: 799-803
- 75 **Carlton JM**, Angiuoli SV, Suh BB, Kooij TW, Perte M, Silva JC, Ermolaeva MD, Allen JE, Selengut JD, Koo HL, Peterson JD, Pop M, Kosack DS, Shumway MF, Bidwell SL, Shal-lom SJ, van Aken SE, Riedmuller SB, Feldblyum TV, Cho JK, Quackenbush J, Sedegah M, Shoaibi A, Cummings LM, Florens L, Yates JR, Raine JD, Sinden RE, Harris MA, Cunningham DA, Preiser PR, Bergman LW, Vaidya AB, van Lin LH, Janse CJ, Waters AP, Smith HO, White OR, Salzberg SL, Venter JC, Fraser CM, Hoffman SL, Gardner MJ, Carucci DJ. Genome sequence and comparative analysis of the model rodent malaria parasite *Plasmodium yoelii yoelii*. *Nature* 2002; **419**: 512-519
- 76 **Gardner MJ**, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen II, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan MS, Nene V, Shallom SJ, Suh B, Peterson J, Angiuoli S, Perte M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DM, Fairlamb AH, Fraunholz MJ, Roos DS, Ralph SA, McFadden GI, Cummings LM, Subramanian GM, Mungall C, Venter JC, Carucci DJ, Hoffman SL, Newbold C, Davis RW, Fraser CM, Barrell B. Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 2002; **419**: 498-511
- 77 **Hall N**, Karras M, Raine JD, Carlton JM, Kooij TW, Berriman M, Florens L, Janssen CS, Pain A, Christophides GK, James K, Rutherford K, Harris B, Harris D, Churcher C, Quail MA, Ormond D, Doggett J, Trueman HE, Mendoza J, Bidwell SL, Rajandream MA, Carucci DJ, Yates JR, Kafatos FC, Janse CJ, Barrell B, Turner CM, Waters AP, Sinden RE. A comprehensive survey of the *Plasmodium* life cycle by genomic, transcriptomic, and proteomic analyses. *Science* 2005; **307**: 82-86
- 78 **Gupta S**, Trenholme K, Anderson RM, Day KP. Antigenic diversity and the transmission dynamics of *Plasmodium falciparum*. *Science* 1994; **263**: 961-963
- 79 **Gupta S**, Swinton J, Anderson RM. Theoretical studies of the effects of heterogeneity in the parasite population on the transmission dynamics of malaria. *Proc Biol Sci* 1994; **256**: 231-238
- 80 **Nair S**, Miller B, Barends M, Jaidee A, Patel J, Mayxay M, Newton P, Nosten F, Ferdig MT, Anderson TJ. Adaptive copy number evolution in malaria parasites. *PLoS Genet* 2008; **4**: e1000243
- 81 **Gonzales JM**, Patel JJ, Ponmee N, Jiang L, Tan A, Maher SP, Wuchty S, Rathod PK, Ferdig MT. Regulatory hotspots in the malaria parasite genome dictate transcriptional variation. *PLoS Biol* 2008; **6**: e238
- 82 **Miller LH**, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. *Nature* 2002; **415**: 673-679
- 83 **Su XZ**, Heatwole VM, Wertheimer SP, Guinet F, Herrfeldt JA, Peterson DS, Ravetch JA, Wellems TE. The large diverse gene family var encodes proteins involved in cytoadherence and antigenic variation of *Plasmodium falciparum*-infected erythrocytes. *Cell* 1995; **82**: 89-100
- 84 **Mayer DC**, Mu JB, Feng X, Su XZ, Miller LH. Polymorphism in a *Plasmodium falciparum* erythrocyte-binding ligand changes its receptor specificity. *J Exp Med* 2002; **196**: 1523-1528
- 85 **Olliaro P**. Drug resistance hampers our capacity to roll back malaria. *Clin Infect Dis* 2005; **41** Suppl 4: S247-S257
- 86 **White NJ**. Antimalarial drug resistance. *J Clin Invest* 2004; **113**: 1084-1092
- 87 **Warhurst DC**. Understanding resistance to antimalarial 4-aminoquinolines, cinchona alkaloids and the highly hydrophobic arylaminoalcohols. *Curr Sci* 2007; **92**: 1556-1560
- 88 **Cooper RA**, Hartwig CL, Ferdig MT. pfcrt is more than the *Plasmodium falciparum* chloroquine resistance gene: a functional and evolutionary perspective. *Acta Trop* 2005; **94**: 170-180
- 89 **Price RN**, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, Patel R, Laing K, Looareesuwan S, White NJ, Nosten F, Krishna S. Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number. *Lancet* 2004; **364**: 438-447
- 90 **Duraisingh MT**, Cowman AF. Contribution of the pfmdr1 gene to antimalarial drug-resistance. *Acta Trop* 2005; **94**: 181-190
- 91 **Gregson A**, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. *Pharmacol Rev* 2005; **57**: 117-145
- 92 **Canfield CJ**, Pudney M, Gutteridge WE. Interactions of atovaquone with other antimalarial drugs against *Plasmodium falciparum* in vitro. *Exp Parasitol* 1995; **80**: 373-381
- 93 **Dondorp AM**, Nosten F, Yi P, Das D, Phyto AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon P, Day NP, Lindegardh N, Socheat D, White NJ. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 2009; **361**: 455-467
- 94 **Dondorp AM**, Yeung S, White L, Nguon C, Day NP, Socheat D, von Seidlein L. Artemisinin resistance: current status and scenarios for containment. *Nat Rev Microbiol* 2010; **8**: 272-280
- 95 **Marfurt J**, Smith TA, Hastings IM, Müller I, Sie A, Oa O, Baisor M, Reeder JC, Beck HP, Genton B. *Plasmodium falciparum* resistance to anti-malarial drugs in Papua New Guinea: evaluation of a community-based approach for the molecular monitoring of resistance. *Malar J* 2010; **9**: 8
- 96 **Herrel N**, Amerasinghe FP, Ensink J, Mukhtar M, van der Hoek W, Konradsen F. Breeding of *Anopheles* mosquitoes in irrigated areas of South Punjab, Pakistan. *Med Vet Entomol* 2001; **15**: 236-248
- 97 **Kusumawathie PH**, Wickremasinghe AR, Karunaweera ND, Wijeyaratne MJ, Yapabandara AM. Anopheline breeding in river bed pools below major dams in Sri Lanka. *Acta Trop* 2006; **99**: 30-33
- 98 **Awolola TS**, Oduola AO, Obansa JB, Chukwurar NJ, Unyimadu JP. *Anopheles gambiae* s.s. breeding in polluted water bodies in urban Lagos, southwestern Nigeria. *J Vector Borne Dis* 2007; **44**: 241-244
- 99 **Graves PM**, Richards FO, Ngondi J, Emerson PM, Shargie EB, Endeshaw T, Ceccato P, Ejigsemahu Y, Mosher AW, Hailemariam A, Zerihun M, Teferi T, Ayele B, Mesele A, Yohannes G, Tilahun A, Gebre T. Individual, household and

- environmental risk factors for malaria infection in Amhara, Oromia and SNNP regions of Ethiopia. *Trans R Soc Trop Med Hyg* 2009; **103**: 1211-1220
- 100 **Dusfour I**, Harbach RE, Manguin S. Bionomics and systematics of the oriental *Anopheles sudaicus* complex in relation to malaria transmission and vector control. *Am J Trop Med Hyg* 2004; **71**: 518-524
- 101 **Obsomer V**, Defourny P, Coosemans M. The *Anopheles dirus* complex: spatial distribution and environmental drivers. *Malar J* 2007; **6**: 26
- 102 **Dash AP**, Adak T, Raghavendra K, Singh OP. The biology and control of malaria vectors in India. *Curr Sci* 2007; **92**: 1571-1578
- 103 **Sharma VP**. Fighting malaria in India. *Curr Sci* 1998; **75**: 1127-1140
- 104 **Subbarao SK**, Raghavendra K. Anopheline species prevalence and prospects for vector control in India. In: Sharma VP, Kirti JS, editors. *Vector Biology: Proceedings of the International Symposium on Vector Biology, Patiala, India, February 18-20, 2006*. India: National Academy of Sciences, 2006
- 105 **Adak T**, Singh OP, Nanda N, Sharma VP, Subbarao SK. Isolation of a *Plasmodium vivax* refractory *Anopheles culicifacies* strain from India. *Trop Med Int Health* 2006; **11**: 197-203
- 106 **Subbarao SK**, Vasantha K, Sharma VP. Responses of *Anopheles culicifacies* sibling species A and B to DDT and HCH in India: implications in malaria control. *Med Vet Entomol* 1988; **2**: 219-223
- 107 **Raghavendra K**, Vasantha K, Subbarao SK, Pillai MK, Sharma VP. Resistance in *Anopheles culicifacies* sibling species B and C to malathion in Andhra Pradesh and Gujarat States, India. *J Am Mosq Control Assoc* 1991; **7**: 255-259
- 108 **Raghavendra K**, Subbarao SK, Vasantha K, Pillai MK, Sharma VP. Differential selection of malathion resistance in *Anopheles culicifacies* A and B (Diptera: Culicidae) in Haryana State, India. *J Med Entomol* 1992; **29**: 183-187
- 109 **Gao Q**, Beebe NW, Cooper RD. Molecular identification of the malaria vectors *Anopheles anthropophagus* and *Anopheles sinensis* (Diptera: Culicidae) in central China using polymerase chain reaction and appraisal of their position within the *Hyrancus* group. *J Med Entomol* 2004; **41**: 5-11
- 110 **Harbach RE**. The classification of genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. *Bull Entomol Res* 2004; **94**: 537-553
- 111 **Bruce-Chwatt LJ**. *Essential Malariology*. 2nd ed. New York: John Wiley and Sons, 1985
- 112 **Levine RS**, Peterson AT, Benedict MQ. Geographic and ecologic distributions of the *Anopheles gambiae* complex predicted using a genetic algorithm. *Am J Trop Med Hyg* 2004; **70**: 105-109
- 113 **Enserink M**. Malaria. Ecologists see flaws in transgenic mosquito. *Science* 2002; **297**: 30-31
- 114 **Besansky NJ**, Powell JR, Caccone A, Hamm DM, Scott JA, Collins FH. Molecular phylogeny of the *Anopheles gambiae* complex suggests genetic introgression between principal malaria vectors. *Proc Natl Acad Sci USA* 1994; **91**: 6885-6888
- 115 **Parmakelis A**, Slotman MA, Marshall JC, Awono-Ambene PH, Antonio-Nkondjio C, Simard F, Caccone A, Powell JR. The molecular evolution of four anti-malarial immune genes in the *Anopheles gambiae* species complex. *BMC Evol Biol* 2008; **8**: 79
- 116 **Akogbeto M**, Romano R. [Infectivity of *Anopheles melas* vis-a-vis *Plasmodium falciparum* in the coastal lagoon area of Benin]. *Bull Soc Pathol Exot* 1999; **92**: 57-61
- 117 **Pock Tsy JM**, Duchemin JB, Marrama L, Rabarison P, Le Goff G, Rajaonarivelo V, Robert V. Distribution of the species of the *Anopheles gambiae* complex and first evidence of *Anopheles merus* as a malaria vector in Madagascar. *Malar J* 2003; **2**: 33
- 118 **Coetzee M**. Distribution of the African malaria vectors of the *Anopheles gambiae* complex. *Am J Trop Med Hyg* 2004; **70**: 103-104
- 119 **Harbach RE**, Townson H, Mukwaya LG, Adeniran T. Use of rDNA-PCR to investigate the ecological distribution of *Anopheles bwambae* in relation to other members of the *An.gambiae* complex of mosquitoes in Bwamba County, Uganda. *Med Vet Entomol* 1997; **11**: 329-334
- 120 **Holt RA**, Subramanian GM, Halpern A, Sutton GG, Charlab R, Nusskern DR, Wincker P, Clark AG, Ribeiro JM, Wides R, Salzberg SL, Loftus B, Yandell M, Majoros WH, Rusch DB, Lai Z, Kraft CL, Abril JF, Anthouard V, Arensburger P, Atkinson PW, Baden H, de Berardinis V, Baldwin D, Benes V, Biedler J, Blass C, Bolanos R, Boscus D, Barnstead M, Cai S, Center A, Chaturverdi K, Christophides GK, Chrystal MA, Clamp M, Cravchik A, Curwen V, Dana A, Delcher A, Dew I, Evans CA, Flanigan M, Grundschober-Freimoser A, Friedli L, Gu Z, Guan P, Guigo R, Hillenmeyer ME, Hladun SL, Hogan JR, Hong YS, Hoover J, Jaillon O, Ke Z, Kodira C, Kokoza E, Koutsos A, Letunic I, Levitsky A, Liang Y, Lin JJ, Lobo NF, Lopez JR, Malek JA, McIntosh TC, Meister S, Miller J, Mobarry C, Mongin E, Murphy SD, O'Brochta DA, Pfannkoch C, Qi R, Regier MA, Remington K, Shao H, Sharakhova MV, Sitter CD, Shetty J, Smith TJ, Strong R, Sun J, Thomasova D, Ton LQ, Topalis P, Tu Z, Unger MF, Walenz B, Wang A, Wang J, Wang M, Wang X, Woodford KJ, Wortman JR, Wu M, Yao A, Zdobnov EM, Zhang H, Zhao Q, Zhao S, Zhu SC, Zhimulev I, Coluzzi M, della Torre A, Roth CW, Louis C, Kalush F, Mural RJ, Myers EW, Adams MD, Smith HO, Broder S, Gardner MJ, Fraser CM, Birney E, Bork P, Brey PT, Venter JC, Weissenbach J, Kafatos FC, Collins FH, Hoffman SL. The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science* 2002; **298**: 129-149
- 121 **Sharakhova MV**, Hammond MP, Lobo NF, Krzywinski J, Unger MF, Hillenmeyer ME, Bruggner RV, Birney E, Collins FH. Update of the *Anopheles gambiae* PEST genome assembly. *Genome Biol* 2007; **8**: R5
- 122 **Beebe NW**, Cooper RD, Foley DH, Ellis JT. Populations of the south-west Pacific malaria vector *Anopheles farauti* s.s. revealed by ribosomal DNA transcribed spacer polymorphisms. *Heredity* (Edinb) 2000; **84** (Pt 2): 244-253
- 123 **Rattanarithikul R**, Harbach RE. *Anopheles maculatus* (Diptera: Culicidae). from the type locality of Hong Kong and two new species of the maculatus complex from the Philippines. *Mosquito System* 1990; **22**: 160-183
- 124 **Somboon P**, Tuno N, Tsuda Y, Takagi M. Evidence of the specific status of *Anopheles flavirostris* (Diptera: Culicidae). *J Med Entomol* 2000; **37**: 476-479
- 125 **Sawabe K**, Takagi M, Tsuda Y, Tuno N. Molecular variation and phylogeny of the *Anopheles minimus* complex (Diptera: Culicidae) inhabiting Southeast Asian countries, based on ribosomal DNA internal transcribed spacers, ITS1 and 2, and the 28S D3 sequences. *Southeast Asian J Trop Med Public Health* 2003; **34**: 771-780
- 126 **Cooper RD**, Waterson DG, Frances SP, Beebe NW, Sweeney AW. Speciation and distribution of the members of the *Anopheles punctulatus* (Diptera: Culicidae) group in Papua New Guinea. *J Med Entomol* 2002; **39**: 16-27
- 127 **Benet A**, Mai A, Bockarie F, Lagog M, Zimmerman P, Alpers MP, Reeder JC, Bockarie MJ. Polymerase chain reaction diagnosis and the changing pattern of vector ecology and malaria transmission dynamics in Papua New Guinea. *Am J Trop Med Hyg* 2004; **71**: 277-284
- 128 **Molina-Cruz A**, de Mérida AM, Mills K, Rodríguez F, Schoua C, Yurrita MM, Molina E, Palmieri M, Black WC. Gene flow among *Anopheles albimanus* populations in Central America, South America, and the Caribbean assessed by microsatellites and mitochondrial DNA. *Am J Trop Med Hyg* 2004; **71**: 350-359
- 129 **Berti J**, Zimmerman R, Amarista J. Adult abundance, biting behavior and parity of *Anopheles aquasalis*, Curry 1932 in

- two malarious areas of Sucre State, Venezuela. *Mem Inst Oswaldo Cruz* 1993; **88**: 363-369
- 130 **Moreno M**, Marinotti O, Krzywinski J, Tadei WP, James AA, Achee NL, Conn JE. Complete mtDNA genomes of *Anopheles darlingi* and an approach to anopheline divergence time. *Malar J* 2010; **9**: 127
- 131 **Vittor AY**, Gilman RH, Tielsch J, Glass G, Shields T, Lozano WS, Pinedo-Cancino V, Patz JA. The effect of deforestation on the human-biting rate of *Anopheles darlingi*, the primary vector of *Falciparum malaria* in the Peruvian Amazon. *Am J Trop Med Hyg* 2006; **74**: 3-11
- 132 **Scarpassa VM**, Tadei WP, Suarez MF. Population structure and genetic divergence in *Anopheles nuneztovari* (Diptera: Culicidae) from Brazil and Colombia. *Am J Trop Med Hyg* 1999; **60**: 1010-1018
- 133 **Manguin S**, Roberts DR, Peyton EL, Rejmankova E, Pecor J. Characterization of *Anopheles pseudopunctipennis* larval habitats. *J Am Mosq Control Assoc* 1996; **12**: 619-626
- 134 **Corbel V**, Chabi J, Dabiré RK, Etang J, Nwane P, Pigeon O, Akogbeto M, Hougard JM. Field efficacy of a new mosaic long-lasting mosquito net (PermaNet 3.0) against pyrethroid-resistant malaria vectors: a multi centre study in Western and Central Africa. *Malar J* 2010; **9**: 113
- 135 **Hemingway J**, Hawkes NJ, McCarroll L, Ranson H. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem Mol Biol* 2004; **34**: 653-665
- 136 **Raghavendra K**, Barik TK, Reddy BP, Sharma P, Dash AP. Malaria vector control: from past to future. *Parasitol Res* 2011; **108**: 757-779
- 137 **Goldberg DE**. Hemoglobin degradation in *Plasmodium*-infected red blood cells. *Semin Cell Biol* 1993; **4**: 355-361
- 138 **Liu J**, Istvan ES, Gluzman IY, Gross J, Goldberg DE. *Plasmodium falciparum* ensures its amino acid supply with multiple acquisition pathways and redundant proteolytic enzyme systems. *Proc Natl Acad Sci USA* 2006; **103**: 8840-8845
- 139 **Gluzman IY**, Francis SE, Oksman A, Smith CE, Duffin KL, Goldberg DE. Order and specificity of the *Plasmodium falciparum* hemoglobin degradation pathway. *J Clin Invest* 1994; **93**: 1602-1608
- 140 **Banerjee R**, Liu J, Beatty W, Pelosof L, Klemba M, Goldberg DE. Four plasmepsins are active in the *Plasmodium falciparum* food vacuole, including a protease with an active-site histidine. *Proc Natl Acad Sci USA* 2002; **99**: 990-995
- 141 **Sijwali PS**, Rosenthal PJ. Gene disruption confirms a critical role for the cysteine protease falcipain-2 in hemoglobin hydrolysis by *Plasmodium falciparum*. *Proc Natl Acad Sci USA* 2004; **101**: 4384-4389
- 142 **Goldberg DE**. Hemoglobin degradation. *Curr Top Microbiol Immunol* 2005; **295**: 275-291
- 143 **Coombs GH**, Goldberg DE, Klemba M, Berry C, Kay J, Mottram JC. Aspartic proteases of *Plasmodium falciparum* and other parasitic protozoa as drug targets. *Trends Parasitol* 2001; **17**: 532-537
- 144 **Sijwali PS**, Shenai BR, Gut J, Singh A, Rosenthal PJ. Expression and characterization of the *Plasmodium falciparum* haemoglobinase falcipain-3. *Biochem J* 2001; **360**: 481-489
- 145 **Sijwali PS**, Kato K, Seydel KB, Gut J, Lehman J, Klemba M, Goldberg DE, Miller LH, Rosenthal PJ. *Plasmodium falciparum* cysteine protease falcipain-1 is not essential in erythrocytic stage malaria parasites. *Proc Natl Acad Sci USA* 2004; **101**: 8721-8726
- 146 **Shenai BR**, Sijwali PS, Singh A, Rosenthal PJ. Characterization of native and recombinant falcipain-2, a principal trophozoite cysteine protease and essential hemoglobinase of *Plasmodium falciparum*. *J Biol Chem* 2000; **275**: 29000-29010
- 147 **Singh N**, Sijwali PS, Pandey KC, Rosenthal PJ. *Plasmodium falciparum*: biochemical characterization of the cysteine protease falcipain-2'. *Exp Parasitol* 2006; **112**: 187-192
- 148 **Drew ME**, Banerjee R, Uffman EW, Gilbertson S, Rosenthal PJ, Goldberg DE. *Plasmodium* food vacuole plasmepsins are activated by falcipains. *J Biol Chem* 2008; **283**: 12870-12876
- 149 **Eggleston KK**, Duffin KL, Goldberg DE. Identification and characterization of falcilysin, a metallopeptidase involved in hemoglobin catabolism within the malaria parasite *Plasmodium falciparum*. *J Biol Chem* 1999; **274**: 32411-32417
- 150 **Klemba M**, Gluzman I, Goldberg DE. A *Plasmodium falciparum* dipeptidyl aminopeptidase I participates in vacuolar hemoglobin degradation. *J Biol Chem* 2004; **279**: 43000-43007
- 151 **Kolakovich KA**, Gluzman IY, Duffin KL, Goldberg DE. Generation of hemoglobin peptides in the acidic digestive vacuole of *Plasmodium falciparum* implicates peptide transport in amino acid production. *Mol Biochem Parasitol* 1997; **87**: 123-135
- 152 **Curley GP**, O'Donovan SM, McNally J, Mullally M, O'Hara H, Troy A, O'Callaghan SA, Dalton JP. Aminopeptidases from *Plasmodium falciparum*, *Plasmodium chabaudi chabaudi* and *Plasmodium berghei*. *J Eukaryot Microbiol* 1994; **41**: 119-123
- 153 **Krugliak M**, Zhang J, Ginsburg H. Intraerythrocytic *Plasmodium falciparum* utilizes only a fraction of the amino acids derived from the digestion of host cell cytosol for the biosynthesis of its proteins. *Mol Biochem Parasitol* 2002; **119**: 249-256
- 154 **Lew VL**, Tiffert T, Ginsburg H. Excess hemoglobin digestion and the osmotic stability of *Plasmodium falciparum*-infected red blood cells. *Blood* 2003; **101**: 4189-4194
- 155 **Atamna H**, Ginsburg H. Origin of reactive oxygen species in erythrocytes infected with *Plasmodium falciparum*. *Mol Biochem Parasitol* 1993; **61**: 231-241
- 156 **Pandey AV**, Singh N, Tekwani BL, Puri SK, Chauhan VS. Assay of beta-hematin formation by malaria parasite. *J Pharm Biomed Anal* 1999; **20**: 203-207
- 157 **Pagola S**, Stephens PW, Bohle DS, Kosar AD, Madsen SK. The structure of malaria pigment beta-haematin. *Nature* 2000; **404**: 307-310
- 158 **Francis SE**, Sullivan DJ, Goldberg DE. Hemoglobin metabolism in the malaria parasite *Plasmodium falciparum*. *Annu Rev Microbiol* 1997; **51**: 97-123
- 159 **Pisciotta JM**, Coppens I, Tripathi AK, Scholl PF, Shuman J, Bajad S, Shulaev V, Sullivan DJ. The role of neutral lipid nanospheres in *Plasmodium falciparum* haem crystallization. *Biochem J* 2007; **402**: 197-204
- 160 **Bonday ZQ**, Taketani S, Gupta PD, Padmanaban G. Heme biosynthesis by the malarial parasite. Import of delta-aminolevulinic acid dehydratase from the host red cell. *J Biol Chem* 1997; **272**: 21839-21846
- 161 **Bonday ZQ**, Dhanasekaran S, Rangarajan PN, Padmanaban G. Import of host delta-aminolevulinic acid dehydratase into the malarial parasite: identification of a new drug target. *Nat Med* 2000; **6**: 898-903
- 162 **Downie MJ**, Kirk K, Mamoun CB. Purine salvage pathways in the intraerythrocytic malaria parasite *Plasmodium falciparum*. *Eukaryot Cell* 2008; **7**: 1231-1237
- 163 **Parker MD**, Hyde RJ, Yao SY, McRobert L, Cass CE, Young JD, McConkey GA, Baldwin SA. Identification of a nucleoside/nucleobase transporter from *Plasmodium falciparum*, a novel target for anti-malarial chemotherapy. *Biochem J* 2000; **349**: 67-75
- 164 **Rager N**, Mamoun CB, Carter NS, Goldberg DE, Ullman B. Localization of the *Plasmodium falciparum* PfNT1 nucleoside transporter to the parasite plasma membrane. *J Biol Chem* 2001; **276**: 41095-41099
- 165 **Downie MJ**, Saliba KJ, Howitt SM, Bröer S, Kirk K. Transport of nucleosides across the *Plasmodium falciparum* parasite plasma membrane has characteristics of PfENT1. *Mol Microbiol* 2006; **60**: 738-748
- 166 **Downie MJ**, Saliba KJ, Bröer S, Howitt SM, Kirk K. Purine nucleobase transport in the intraerythrocytic malaria parasite. *Int J Parasitol* 2008; **38**: 203-209
- 167 **Quashie NB**, Dorin-Semblat D, Bray PG, Biagini GA, Doerig

- C, Ranford-Cartwright LC, De Koning HP. A comprehensive model of purine uptake by the malaria parasite *Plasmodium falciparum*: identification of four purine transport activities in intraerythrocytic parasites. *Biochem J* 2008; **411**: 287-295
- 168 **Kirk K**, Howitt SM, Bröer S, Saliba KJ, Downie MJ. Purine uptake in *Plasmodium*: transport versus metabolism. *Trends Parasitol* 2009; **25**: 246-249
- 169 **Riegelhaupt PM**, Cassera MB, Fröhlich RF, Hazleton KZ, Hefter JJ, Schramm VL, Akabas MH. Transport of purines and purine salvage pathway inhibitors by the *Plasmodium falciparum* equilibrative nucleoside transporter PfENT1. *Mol Biochem Parasitol* 2010; **169**: 40-49
- 170 **El Bissati K**, Downie MJ, Kim SK, Horowitz M, Carter N, Ullman B, Ben Mamoun C. Genetic evidence for the essential role of PfNT1 in the transport and utilization of xanthine, guanine, guanosine and adenine by *Plasmodium falciparum*. *Mol Biochem Parasitol* 2008; **161**: 130-139
- 171 **Webster HK**, Whaun JM. Purine metabolism during continuous erythrocyte culture of human malaria parasites (*P. falciparum*). *Prog Clin Biol Res* 1981; **55**: 557-573
- 172 **Reyes P**, Rathod PK, Sanchez DJ, Mrema JE, Rieckmann KH, Heidrich HG. Enzymes of purine and pyrimidine metabolism from the human malaria parasite, *Plasmodium falciparum*. *Mol Biochem Parasitol* 1982; **5**: 275-290
- 173 **Ting LM**, Shi W, Lewandowicz A, Singh V, Mwakingwe A, Birck MR, Ringia EA, Bench G, Madrid DC, Tyler PC, Evans GB, Furneaux RH, Schramm VL, Kim K. Targeting a novel *Plasmodium falciparum* purine recycling pathway with specific immucillins. *J Biol Chem* 2005; **280**: 9547-9554
- 174 **Padmanaban G**, Nagaraj VA, Rangarajan PN. Drugs and drug targets against malaria. *Curr Sci* 2007; **92**: 1545-1555
- 175 **Hyde JE**. Exploring the folate pathway in *Plasmodium falciparum*. *Acta Trop* 2005; **94**: 191-206
- 176 **Chulay JD**, Watkins WM, Sixsmith DG. Synergistic antimalarial activity of pyrimethamine and sulfadoxine against *Plasmodium falciparum* in vitro. *Am J Trop Med Hyg* 1984; **33**: 325-330
- 177 **Phillips MA**, Rathod PK. *Plasmodium* dihydroorotate dehydrogenase: a promising target for novel anti-malarial chemotherapy. *Infect Disord Drug Targets* 2010; **10**: 226-239
- 178 **Wrenger C**, Knöckel J, Walter RD, Müller IB. Vitamin B1 and B6 in the malaria parasite: requisite or dispensable? *Braz J Med Biol Res* 2008; **41**: 82-88
- 179 **Müller IB**, Hyde JE, Wrenger C. Vitamin B metabolism in *Plasmodium falciparum* as a source of drug targets. *Trends Parasitol* 2010; **26**: 35-43
- 180 **Wrenger C**, Eschbach ML, Müller IB, Warnecke D, Walter RD. Analysis of the vitamin B6 biosynthesis pathway in the human malaria parasite *Plasmodium falciparum*. *J Biol Chem* 2005; **280**: 5242-5248
- 181 **Wrenger C**, Eschbach ML, Müller IB, Laun NP, Begley TP, Walter RD. Vitamin B1 de novo synthesis in the human malaria parasite *Plasmodium falciparum* depends on external provision of 4-amino-5-hydroxymethyl-2-methylpyrimidine. *Biol Chem* 2006; **387**: 41-51
- 182 **Gengenbacher M**, Fitzpatrick TB, Raschle T, Flicker K, Sinning I, Müller S, Macheroux P, Tews I, Kappes B. Vitamin B6 biosynthesis by the malaria parasite *Plasmodium falciparum*: biochemical and structural insights. *J Biol Chem* 2006; **281**: 3633-3641
- 183 **Foth BJ**, McFadden GI. The apicoplast: a plastid in *Plasmodium falciparum* and other Apicomplexan parasites. *Int Rev Cytol* 2003; **224**: 57-110
- 184 **Ralph SA**, van Dooren GG, Waller RF, Crawford MJ, Fraunholz MJ, Foth BJ, Tonkin CJ, Roos DS, McFadden GI. Tropical infectious diseases: metabolic maps and functions of the *Plasmodium falciparum* apicoplast. *Nat Rev Microbiol* 2004; **2**: 203-216
- 185 **Upadhyay SK**, Misra A, Srivastava R, Surolia N, Surolia A, Sundt M. Structural insights into the acyl intermediates of the *Plasmodium falciparum* fatty acid synthesis pathway: the mechanism of expansion of the acyl carrier protein core. *J Biol Chem* 2009; **284**: 22390-22400
- 186 **Ben Mamoun C**, Prigge ST, Vial H. Targeting the Lipid Metabolic Pathways for the Treatment of Malaria. *Drug Dev Res* 2010; **71**: 44-55
- 187 **Tarun AS**, Vaughan AM, Kappe SH. Redefining the role of de novo fatty acid synthesis in *Plasmodium* parasites. *Trends Parasitol* 2009; **25**: 545-550
- 188 **Krungkrai J**, Prapunwattana P, Krungkrai SR. Ultrastructure and function of mitochondria in gametocytic stage of *Plasmodium falciparum*. *Parasite* 2000; **7**: 19-26
- 189 **van Dooren GG**, Stimmmer LM, McFadden GI. Metabolic maps and functions of the *Plasmodium* mitochondrion. *FEMS Microbiol Rev* 2006; **30**: 596-630
- 190 **Patel AP**, Staines HM, Krishna S. New antimalarial targets: the example of glucose transport. *Travel Med Infect Dis* 2008; **6**: 58-66
- 191 **Prabhu P**, Patravale V. Novel targets for malaria therapy. *Curr Drug Targets* 2011; **12**: 2129-2143
- 192 **Staines HM**, Ellory JC, Chibale K. The new permeability pathways: targets and selective routes for the development of new antimalarial agents. *Comb Chem High Throughput Screen* 2005; **8**: 81-88
- 193 **Waters NC**, Geyer JA. Cyclin-dependent protein kinases as therapeutic drug targets for antimalarial drug development. *Expert Opin Ther Targets* 2003; **7**: 7-17
- 194 **McConkey GA**. Targeting the shikimate pathway in the malaria parasite *Plasmodium falciparum*. *Antimicrob Agents Chemother* 1999; **43**: 175-177
- 195 **Kun JF**, de Carvalho EG. Novel therapeutic targets in *Plasmodium falciparum*: aquaglyceroporins. *Expert Opin Ther Targets* 2009; **13**: 385-394

S- Editor Song XX L- Editor Hughes D E- Editor Xiong L



Acknowledgments to reviewers of World Journal of Translational Medicine

We acknowledge our sincere thanks to our reviewers. Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of our World Series Journals. Both the editors of the journals and authors of the manuscripts submitted to the journals are grateful to the following reviewers for reviewing the articles (either published or rejected) over the past period of time.

Ahmet Gokce, MD, Assistant Professor, Department of Urology, Tulane University - Health Sciences Center, 1430 Tulane Avenue, SL-42, New Orleans, LA 70112, United States

Georg F Weber, MD, PhD, Associate Professor, James L. Winkle College of Pharmacy, University of Cincinnati Academic Health Center, 3225 Eden Avenue, Cincinnati, OH 45267-0004, United States

Ruggero Ridolfi, MD, Director, Immunotherapy and Somatic Cell Therapy Unit, Romagna Cancer Institute, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Via Piero Maroncelli, 40 - 47014 Meldola, Italy

Luis Huicho, Professor, Department of Pediatrics, Universidad Peruana Cayetano Heredia, Batallon Libres de Trujillo 227, L133, Lima, Peru

Events Calendar 2012

March 19-21, 2012
2012 Summit on Translational Bioinformatics
San Francisco, CA, United States

May 7-8, 2012
LIPID MAPS Meeting 2012 - LIPID MAPS Annual Meeting 2012: Impact on Cell Biology, Metabolomics and Translational Medicine
La Jolla, CA, United States

May 10-11, 2012
4th Annual Translational Medicine Conference
City Hotel, Derry/Londonderry, Northern Ireland

May 11-12, 2012
Integrative Network Biology 2012: Network Medicine
Helsingør, Denmark

May 23-25, 2012
Association for Cancer Immunotherapy (CIMT) Annual Meeting 2012
10th CIMT Annual Meeting
Mainz, Germany

June 11-12, 2012
The Lung - a Pivotal Player in Systemic Inflammatory Disorders
Solna, Sweden

June 28-29, 2012
Worldwide Innovative Networking (WIN) Consortium WIN 2012 Symposium
Paris, France

July 13-14, 2012
13th Annual Bioinformatics Open Source Conference

Long Beach, CA, United States

September 13-15, 2012
2012 Breast Cancer Symposium
San Francisco, CA, United States

September 17-19, 2012
International Conference on Translational Medicine
Holiday Inn San Antonio, TX, United States

September 20-22, 2012
Temporomandibular Joint Disorder Bioengineering Conference
Pittsburgh, PA, United States

October 11-13, 2012
Markers in Cancer 2012
Holywood, FL, United States

October 12-14, 2012
European Society for Organ Transplantation-American Society of Transplantation Joint Meeting: Transformational Therapies and Diagnostics in Transplantation
Nice, France

October 14-17, 2012
Current and Future Applications of Focused Ultrasound 2012
Washington DC, United States

November 7-9, 2012
3rd AMA-IEEE Medical Technology Conference: Translational Engineering in Health and Medicine: the Intersection of the Lab, the Clinic and the Community
Texas, United States

November 16-18, 2012
Conference on Instrumentations and Biotechnologies 2012
Shanghai, China

GENERAL INFORMATION

World Journal of Translational Medicine (*World J Transl Med*, *WJTM*, online ISSN 2220-6132, DOI: 10.5528) is a bimonthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 103 experts in translational medicine from 27 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJTM* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJTM* is an OA journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJTM* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles,

thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

The aim of *WJTM* is to report rapidly new theories, methods and techniques for prevention, diagnosis, treatment, rehabilitation and nursing in the field of translational medicine, focusing on information derived from human experimentation so as to optimize the communication between basic and clinical science. *WJTM* covers animal and human studies, applied physical sciences, behavior, bio-engineering, biomarkers, cancer, cardiovascular disease, cell culture, chemical genomics/drug discovery, data mining, drug delivery, gene therapy/regenerative medicine, imaging, immunology/vaccines, infectious diseases, medical informatics, medical nanotechnology, metabolism/diabetes/obesity, neuroscience/neurology/psychiatry, other interdisciplinary approaches to medicine, physics, policy, toxicology and pharmacokinetics, and translational medicine-related traditional medicine, integrated Chinese and Western medicine. The journal also publishes original articles and reviews that report the results of translational medicine-related applied and basic research in fields such as immunology, physiopathology, cell biology, pharmacology, medical genetics, and pharmacology of Chinese herbs.

Columns

The columns in the issues of *WJTM* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide Guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Articles: To report innovative and original findings in translational medicine; (9) Brief Articles: To briefly report the novel and innovative findings in translational medicine; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJTM*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of translational medicine; and (13) Guidelines: To introduce consensus and guidelines reached by international and national academic authorities worldwide on the research translational medicine.

Name of journal

World Journal of Translational Medicine

ISSN

ISSN 2220-6132 (online)

Editor-in-Chief

Alfonso Dueñas-Gonzalez, MD, PhD, Unit of Biomedical Re-

Instructions to authors

search on Cancer, Instituto de Investigaciones Biomédicas, UNAM, Instituto Nacional de Cancerología. Primer piso, edificio de Investigación, San Fernando 22, Tlalpan 14080, Mexico

Ruggero Ridolfi, MD, Director, Immunotherapy and Somatic Cell Therapy Unit, Romagna Cancer Institute, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Via Piero Maroncelli, 40 - 47014 Meldola, Italy

Editorial Office

World Journal of Translational Medicine
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjtm@wjnet.com
http://www.wjnet.com
Telephone: +86-10-85381891
Fax: +86-10-8538-1893

Indexed and Abstracted in

Digital Object Identifier.

Published by

Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing

Statistical review is performed after peer review. We invite an expert in Biomedical Statistics from to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc.* The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement

In the interests of transparency and to help reviewers assess any potential bias, *WJTM* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent

Manuscripts should contain a statement to the effect that all human

studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights

When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is <http://www.clinicaltrials.gov> sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: <http://www.wjnet.com/2220-6132/office>. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjnet.com/2220-6132/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online

Submission System may send an email describing the problem to wjtm@wjgnet.com, or by telephone: +86-10-85381891. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJTM*, review-

ers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no less than 256 words) and structured abstracts (no less than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no less than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/...; MATERIALS AND METHODS (no less than 140 words); RESULTS (no less than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 vs 3.61 ± 1.67 , $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/2220-6132/g_info_20100725072755.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: <http://www.wjgnet.com/1007-9327/13/4520.pdf>; <http://www.wjgnet.com/1007-9327/13/4554.pdf>; <http://www.wjgnet.com/1007-9327/13/4891.pdf>; <http://www.wjgnet.com/1007-9327/13/4986.pdf>; <http://www.wjgnet.com/1007-9327/13/4498.pdf>. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. ^a*P* < 0.05, ^b*P* < 0.01 should be noted (*P* > 0.05 should not be noted). If there are other series of *P* values, ^c*P* < 0.05 and ^d*P* < 0.01 are used. A third series of *P* values can be expressed as ^e*P* < 0.05 and ^f*P* < 0.01. Other notes in tables or under illustrations should be expressed as ¹F, ²F, ³F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, etc., in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability^[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references^[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at <http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed> and <http://www.crossref.org/SimpleTextQuery/>, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic

effect of Jianpi Yishen decoction in treatment of Pixu-diarhoea. *Shijie Huaren Xiaobua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRS A Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wiczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/eid/index.htm>

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean \pm SD or mean \pm SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as *v* (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, *m* (B) = 78 kg; blood pressure, *p* (B) = 16.2/12.3 kPa; incubation time, *t* (incubation) = 96 h; blood glucose concentration, *c* (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, *p* (CEA) = 8.6 24.5 μ g/L; CO₂ volume fraction, 50 mL/L CO₂, not 5% CO₂; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, etc. Arabic numerals such as 23, 243, 641 should be read 23243641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2220-6132/g_info_20100725073806.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

Genotypes: *gyrA*, *arg 1*, *c myc*, *c fos*, etc.

Restriction enzymes: *EcoRI*, *HindI*, *BamHI*, *Kbo I*, *Kpn I*, etc.

Biology: *H. pylori*, *E. coli*, etc.

Examples for paper writing

Editorial: http://www.wjgnet.com/2220-6132/g_info_20100725071851.htm

Frontier: http://www.wjgnet.com/2220-6132/g_info_20100725071932.htm

Topic highlight: http://www.wjgnet.com/2220-6132/g_info_20100725072121.htm

Observation: http://www.wjgnet.com/2220-6132/g_info_20100725072232.htm

Guidelines for basic research: http://www.wjgnet.com/2220-6132/g_info_20100725072344.htm

Guidelines for clinical practice: http://www.wjgnet.com/2220-6132/g_info_20100725072543.htm

Review: http://www.wjgnet.com/2220-6132/g_info_20100725072656.htm

Original articles: http://www.wjgnet.com/2220-6132/g_info_20100725072755.htm

Brief articles: http://www.wjgnet.com/2220-6132/g_info_20100725072920.htm

Case report: http://www.wjgnet.com/2220-6132/g_info_20100725073015.htm

Letters to the editor: <http://www.wjgnet.com/2220-6132/>

[g_info_20100725073136.htm](http://www.wjgnet.com/2220-6132/g_info_20100725073136.htm)

Book reviews: http://www.wjgnet.com/2220-6132/g_info_20100725073214.htm

Guidelines: http://www.wjgnet.com/2220-6132/g_info_20100725073300.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJTM*. The revised version including manuscript and high-resolution image figures (if any) should be re-submitted online (<http://www.wjgnet.com/2220-6132office/>). The author should send the copyright transfer letter, responses to the reviewers, English language Grade B certificate (for non-native speakers of English) and final manuscript checklist to wjtm@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2220-6132/g_info_20100725073726.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2220-6132/g_info_20100725073445.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJTM will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (<http://www.eurekalert.org>). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

WJTM is an international, peer-reviewed, OA, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. The related standards are as follows. Publication fee: 1300 USD per article. Editorial, topic highlights, original articles, brief articles, book reviews and letters to the editor are published free of charge.