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Plasmodium vivax malaria: challenges in diagnosis, treatment, and elimination

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Introduction:

P. vivax (Pv) is the second major cause of malaria, after *P. falciparum* (Pf). There is an increasing recognition that Pv can be associated with severe disease and serious complications in pregnancy, and carries a major health, social and economic burden. Diagnosis and clinical management of Pv malaria can be difficult, and the control and elimination of Pv presents special challenges.

After inoculation by Anopheles mosquitoes, sporozoites migrate to the liver and infect hepatocytes. Over 7-10 days, parasites develop and divide into merozoites that are released into the bloodstream where they invade reticulocytes and replicate inside them, commencing the (48 hour) blood-stage of infection. Clinical illness develops during the blood-stage and the great majority of drugs target this developmental stage. An important feature of Pv, which differs from Pf, is the occurrence of dormant hypnozoites in the liver that can reactivate weeks, months or years later to initiate new episodes of blood-stage infection, presenting a major challenge to treatment, control and elimination.

Epidemiology and clinical features

Pv has a wide geographical distribution with an estimated 2.5 billion individuals at risk[1]. The wider distribution of Pv than Pf may be due to hypnozoites and better survival in Anopheles at lower temperatures and higher altitudes. The greatest burden is in central Asia (82%); Southeast Asia (9%), the Americas (6%), and Africa (3%). Most of those at risk (~1.5 billion) live in areas of unstable transmission where risk of Pv infection is very low. Estimates of total Pv infections range from 71 to 391 million infections/year[2]. It is also unclear how many Pv infections progress to clinical disease as afebrile parasitemia is common, but studies suggest that Pv infection and illness are more common in children and pregnant women than non-pregnant adults[3,4]. The low prevalence of Pv in Africa is attributed to the high frequencies of Duffy negativity, which renders individuals largely refractory to Pv;[5] Duffy antigen is an important receptor for merozoite invasion of reticulocytes. Southeast Asian ovalocytosis may also protect against Pv infection and clinical disease[6].

The clinical presentation in children with uncomplicated Pv malaria varies depending on age and cannot be easily differentiated from other infectious diseases[7]. A high index of suspicion is necessary for diagnosis in low transmission settings. In all age groups, symptomatic infections commonly cause fever, chills and headache; in infants fever may be the only

symptom. Pv typically presents with low parasitemia and appears to cause fever and symptoms at a lower parasitemia than Pf. Other symptoms may include cough and abdominal pain; occasionally there may be associated respiratory distress or diarrhoea. In pregnant women, Pv results in low birth weight[4]. Possible long-term effects of infection in pregnancy are not well understood.

Recent studies have highlighted Pv as a cause of severe illness, most commonly associated with coma or convulsions, respiratory distress and severe anaemia[7]. However, it remains unclear to what extent Pv directly contributes to severe morbidity, and other possible causes of the clinical presentation need to be investigated before a diagnosis of severe malaria is made (E.g. anaemia, meningitis, sepsis, scrub typhus, dengue fever or other viruses, or co-infection with Pf). There are increasing reports of ARDS, myocarditis, acute renal failure, glomerulonephritis, hepatitis, severe thrombocytopenia and pancytopenia in association with Pv. The effects of chronic relapsing Pv infection due to dormant hypnozoites are extensive and debilitating, and include chronic anemia.[7,8] The triggers for relapse are poorly understood.

Diagnosis and treatment

Pv preferentially infects reticulocytes, which limits parasite densities, and therefore diagnostic tests need to have high sensitivity. Microscopy remains the gold standard and performs well when conducted by skilled technicians, but remains a challenge in resource-poor settings. It is difficult to implement in mass treatment programs where large numbers of tests are needed, or where Pv prevalence is very low. Rapid diagnostic tests (RDTs), based on Plasmodium antigen detection, are available and have the advantage of bedside or community-based testing. However, sensitivity is sub-optimal, particularly for low parasite densities, and tests may remain positive for days after clearance of parasitemia by antimalarials[9]. Many RDTs are unable to distinguish mixed Pf-Pv infections, which are common. PCR-based methods have better sensitivity and specificity, but their use is limited to well-resourced laboratories. Methods such as loop-mediated isothermal amplification (LAMP) may provide simpler, cheaper and more portable tests with increased sensitivity.[10]

Standard treatment of Pv is a 3-day course of chloroquine. Resistance to chloroquine was first reported in 1989 in Papua New Guinea and Indonesia, and has since been identified in 12 countries.[11] In settings with high-grade resistance, dihydroartemisinin-piperaquine or artemether-lumefantrine are generally used. Primaquine is the only drug available and

licensed to treat hypnozoites, and is usually given for 14 days. Primaquine can cause hemolysis in glucose-phosphate-dehydrogenase (G6PD)-deficient individuals, and deficiency is common in many malaria-endemic regions. Ideally, testing for G6PD deficiency should be performed prior to use of primaquine[12], especially in children. Widespread use of primaquine is limited by a lack of availability of G6PD testing and the inability to supervise 14-day treatment. Primaquine should not be administered to pregnant women as the G6PD status of the foetus cannot be determined. Data on the effects of primaquine in infants are scarce; current recommendation is to avoid its use in children <4 years, particularly infants[13]. The development of shorter courses, such as high-dose primaquine over seven days, or tafenoquine (currently in trials) may increase compliance. There are a range of G6PD tests available[12], but most pose technical and financial challenges in resource-poor settings. The gold standard test is a quantitative UV-spectrophotometry test that requires specialized equipment and is costly. The only WHO-approved point-of-care test is the fluorescent spot test, which is also expensive and requires laboratory facilities. Simpler point-of-care tests have become available, but have significant limitations for widespread implementation[12]. G6PD-deficiency is an X-linked heritable condition; females may manifest a heterozygous genotype in which only a proportion of the erythrocytes are G6PD-deficient making it difficult to accurately assess the level of deficiency[12]

Immunity and vaccines

The incidence of symptomatic Pv-malaria and Pv density and prevalence decreases with age, reflecting the acquisition of immunity from repeated exposure[5], and antibodies play a major role. Antibody targets include antigens expressed on the invading merozoite and the surface of infected erythrocytes (erythrocytic stage), sporozoites, and possibly hypnozoites. There is a paucity of studies investigating immunity to Pv, and few studies have been conducted in the Asia-Pacific region despite representing most of the global Pv burden[14]. Studies have been hindered by technical challenges in maintaining *in vitro* cultures of Pv, and limitations of animal models. Assumptions of Pv immunity have often been based on studies of Pf or primate malarias. Cell-mediated immunity may play a role, particularly for liver-stage infection, but there is a lack of evidence in humans.

Successful control and elimination of Pv may depend on an effective vaccine that protects against clinical illness and reduces transmission. Vaccine approaches could target each of the stages of the Pv life-cycle. Those that result in antibodies against sporozoites, or in cell-

mediated immunity against infected hepatocytes, aim to prevent establishment of blood-stage infection by preventing infection of hepatocytes. Vaccines targeting merozoite antigens aim to inhibit parasite replication and clear parasites from the circulation to prevent clinical disease. Transmission-blocking vaccines aim to prevent transmission of infection to mosquitoes by targeting gametocytes, the transmissible blood-stage form, or other stages in mosquitoes. This would not directly prevent clinical illness, but inclusion in a multi-antigen vaccine may facilitate malaria elimination. Very few Pv vaccine candidates have progressed into clinical trials, and no phase 2 field efficacy trials have been published (http://www.who.int/immunization/research/development/Rainbow_tables/en/). However, several promising candidates are being advanced. In contrast, many more Pf vaccines have progressed into phase 1 and 2 clinical trials, and the RTS,S vaccine is in phase 3 trials. Although these will not protect against Pv, lessons learned from these trials will be valuable for advancing Pv vaccines[15].

Challenges for elimination

There has been a renewed impetus in malaria elimination programs, with eradication as the long-term goal. There has been a major reduction in malaria globally over the last decade, through established interventions of long-lasting insecticide-treated bed nets, improved diagnosis and treatment, insecticides, and intermittent preventive-treatment in some settings. However, there are significant challenges for control of Pv (summarized in Table 1), and elimination cannot be achieved with current approaches. Where Pf and Pv malaria co-exist, the impact of established control interventions has often been greater for Pf. Detection and clearance of hypnozoite carriage is a major challenge, as failure to clear hypnozoites in populations will result in ongoing transmission, and migrant populations may carry infections into disease-free regions. There is no population screening test for hypnozoites and it is not possible to identify asymptomatic carriers of hypnozoites. Better knowledge of epidemiology and strengthened surveillance systems and tools may enable the development of strategies for identifying and targeting high risk groups. Sub-optimal sensitivity of diagnostics is a further significant barrier to population screening and treatment in elimination programs. Tests that are sensitive enough to detect asymptomatic Pv infections, and reliable tests for G6PD-deficiency prior to administering primaquine would facilitate enhanced control and elimination.

Future

A renewed global effort against Pv, including research and programmatic activities, raises hopes that ongoing progress in control will be achieved in the coming decade. However, there are many urgent needs in order to achieve more effective control and, ultimately, elimination in many regions. New drugs to replace primaquine that do not cause haemolysis, improving primaquine treatment regimens for compliance, tests for G6PD-deficiency, more sensitive and specific RDTs, and greater knowledge of epidemiology and transmission would greatly aid control and elimination efforts. In the longer term, an effective vaccine could be transformative for control and elimination efforts.

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Table 1: Challenges and priorities for the control and elimination of *P. vivax* malaria

Priority issues
Diagnostics
More sensitive and specific diagnostic tests
G6PD deficiency tests that are point-of-care, cheap, and suitable for resource-poor settings
Development and implementation of nucleic acid detection methods for diagnosis and screening
Tests for the detection of hypnozoites
Treatment and clinical
Short-course therapies for clearance of hypnozoites
New drugs for clearance of hypnozoites that are not dependent on knowing G6PD status
Markers for chloroquine resistance
Alternative therapies for CQ resistance
Single drug regimen for use with Pf and Pv
Safe treatment of liver-stage and blood-stage Pv for pregnant women and children
Better understanding of the capacity of Pv to cause severe malaria and its clinical features
Control and elimination
Strategies or tests to identify ongoing transmission and hypnozoite carriage
Ability to distinguish new infections from relapses
Greater understanding of the epidemiology of Pv to support effective elimination programs
Sensitive and low-cost tests suitable for mass screening for Pv infection
Integrated strategies for effective interruption of transmission
Optimization of mass drug treatment strategies
Continued surveillance for drug resistance
Vaccines and immunity
Greater understanding of immunity: targets and mechanisms, acquisition and maintenance
Fast-tracking vaccine candidates into phase 1 and 2 clinical trials
Development of new vaccine candidates and Pf-Pv combinations vaccines
Immune correlates of protection for vaccine trials and population monitoring
Biology
Improved capacity for long term culture of Pv
Greater knowledge of disease pathogenesis