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Focus
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Diagnosis of Malaria

Malaria

The clinical practice recommendations from the consensus conference in 2007 (http://www.infectiologie.com/site/medias/_documents/consensus/2007-paludisme-court.pdf) are designed to diagnose malaria as early as possible: i.e. in the case of fever in patients who have returned from a visit to an endemic zone (including those who followed chemoprophylactic treatment). Even in cases with low clinical suspicion, a serum sample needs to be taken and thick and thin blood smears need to be made in order to screen for Plasmodium antigen and to perform microscopy for malarial parasites. Testing should be repeated in 24 to 48 hours if the first result is negative and yet the fever persists, especially for those who have an extended hospitalization period (such as orthopaedic patients or those in intensive care).

Diagnosis and treatment are now standardised:
"In the case of a strong epidemiological and clinical suspicion of malaria in a patient showing signs of deterioration, treatment should be given straight away and should not be delayed due to the lack of an emergency parasitological diagnosis (blood smear and thick blood smear)..In all cases, parasitology confirmation should be obtained as quickly as possible."

Symptoms

The incubation period is on average 15 days. A history of mosquito bites is present in over 90% of cases. The first symptoms surface after at least 7 days following the bite, and can sometimes appear even several months later. The fever grows progressively, and is poorly tolerated, resistant to antipyretics, accompanied by headaches, a flu-like syndrome, vomiting and digestive upset. Severe malaria is defined by rapid deterioration of the state of the patient, with cerebral symptoms, respiratory distress and multiple organ failure. Pernicious malaria is characterized by neurological disorders followed by a coma that requires a rapid transfer to Intensive Care and is associated with a high mortality rate if treatment is not given straight away.

Clinical diagnosis of malaria

The clinical diagnosis of malaria requires as a minimum, the patient's clinical details including any details on trips abroad. A full blood count can be indicative if thrombopenia ‹150,000/mm3 and/or on rare occasions, anaemia are present.

Thin and thick blood smears must be performed along with (if possible) a Plasmodium rapid antigen detection test. When not in an emergency situation PCR testing can be useful in the diagnosis of low parasitemia cases and mixed infections. Serology testing is only used in the retrospective diagnosis of a P.falciparum infection.

Thin blood smear

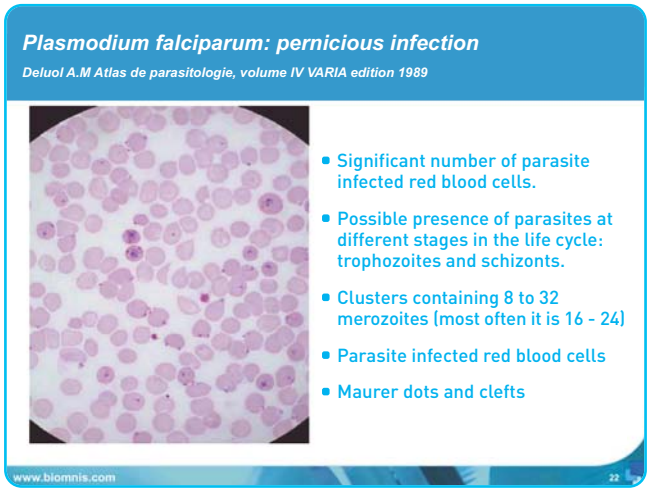
- Smear a fine, erythrocyte monolayer
- Dry and fix with methanol
- Stain using May Grunwald Giemsa (MGG) and/or via a rapid technique (RAL 555R, HemacolorR or DiffquickR)
- Read 200 to 300 fields in submersion (i.e. 0.005 to 0.01µl of blood)
- Results released within 1 hour following the sample collection: species, predominant parasite life-cycle stage, parasitemia (% of parasitized red blood cells/ total red blood cells or number of parasites per micro litre of total blood).

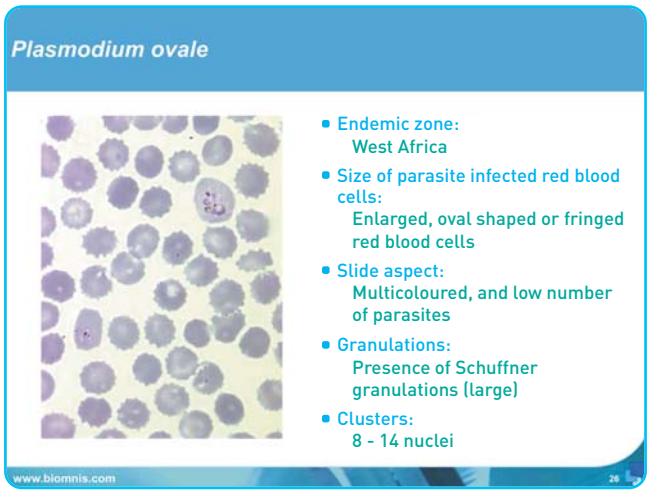
Thick blood smear

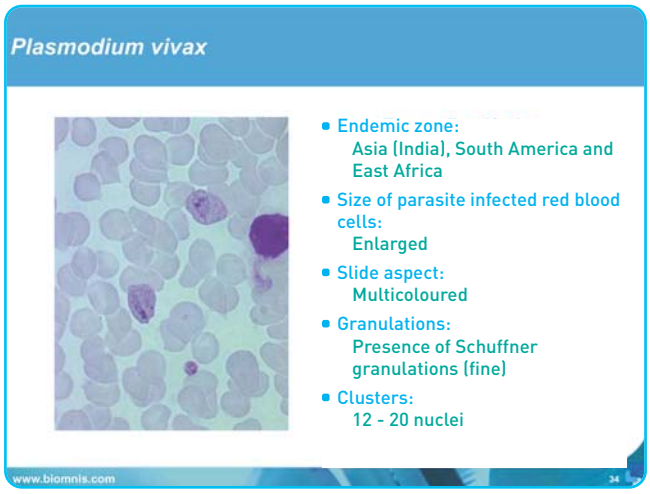
- Smear blood on the slide and remove fibrin
- Once dried, haemolyse via a Giemsa 3% solution in a phosphate buffer at pH 7.2 or via the quicker Thellier method (10 minutes with a formaldehyde and saponin based reagent)
- Read 200 fields, i.e. approximately 1µl for 10 minutes
- Sensitivity: approximately 5 parasites/µl.

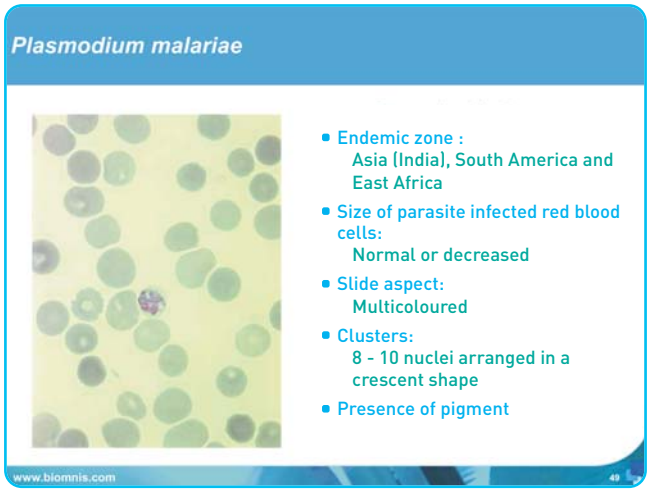
Repeat testing of parasitemia during treatment

This is obligatory in severe cases (i.e. parasitemia ›4%, or ›20% in an immune subject) and must be performed on the third or fourth day after infection, and repeated as needed during days 7 to 9, and then during days 28 to 30. Using the blood slides, parasitemia must be calculated following examination of at least 10,000 red blood cells (i.e. 30 fields) or for thick blood smears the parasite percentage must be determined per 100 leucocytes (less precise).
Therapeutic failure can be inferred if parasitemia remains static or increases at over 36 hours from treatment (especially when returning from trips to Asia).









Rapid parasite antigen detection tests

Rapid parasite antigen detection tests are diagnostic tools, but do not replace testing by thin and thick blood smears.

The Binax Now® Malaria test

The Binax Now® Malaria test can detect the HRP-2 protein or Histidine Rich Protein secreted by hematozoa and is found on the cell surface of parasite-infected red blood cells. It is highly sensitive to P. falciparum (96 % in comparison to PCR) and less so for P. vivax (87 %) and insufficiently sensitive to P. malariae and P. ovale (67 %).
The HRP-2 antigenemia can persist for 15 - 30 days after infection by the malaria parasite (even if the blood slide results are negative or if only gametocytes are present). Its detection therefore allows us to make an a posteriori diagnosis.

In practice, its implementation is quick, easy and requires no specific material. There are no false positives (the rheumatoid factor does not interfere). If the blood smear is read as negative with a positive rapid test result, it could be a low parasitmeia that requires thick and thin blood smears to be reread and hence underlines the significance of these two tests in combination. HRP-2 false negatives are rare with P. falciparum (a little less with the other species (see below)).

The OptiMAL-IT® test

This test analyses pfHRP-2 as well as pLDH. pLDH is an enzyme secreted by all the human plasmodia in the middle of intra-erythrocyte development. The enzyme disappears from the blood at the same time as the parasites and so is negative under treatment that works on HRP-2. The sensitivity of pLDH is 92.6% for the detection of Plasmodium falciparum.

Airport malaria

Airport malaria is caused by the Anopheles genus of mosquitoes which are infected by a Plasmodium and travel via airplane from an endemic zone to a zone that is non-endemic for malaria. It is rare (since 1969, 30 notified cases, 2 of which were notified during the summer of 2008) but all the same serious due to the frequently delayed diagnosis of unprotected individuals. In France it is a disease that must be notified. The International Health Regulation imposes on all states that all airplanes coming from malarial or arbovirus endemic zones must be systematically checked and pest controlled. Anti-vectoral measures must be in force within a radius of at least 400 meters of airport perimeters in malarial or arboviral endemic zones.

Conclusion

Close cooperation between clinician and pathologist is essential in the diagnosis of malaria. One must not forget to check slides for patients coming back from locations below the tropics and who present with an infection that does not respond to antibiotics.

Carole Emile, from a report by Jacques Yves Nizou, Biomnis Paris.