Malaria – Diagnosis

General

When can one assert that someone has the disease “malaria”? There are several problems and the question has still not been fully resolved. The demonstration of malaria parasites in the blood is essential but insufficient in itself. Most cases are accompanied by thrombocytopenia and normal white count. Many people will develop an acquired immunity after several years of exposure and may harbour parasites without exhibiting symptoms. The degree of parasitaemia may help, but there is no absolute criterion (the higher the parasitaemia, the more chance that malaria is in fact the diagnosis). There are patients with malaria for whom the thick smear is negative (luckily this is rare in a good laboratory). There are no pathognomonic clinical signs. An accurate diagnosis is becoming more and more important, in view of the increasing resistance of *P. falciparum* and the higher price of modern combinationtreatments.

Clinical aspects

No single clinical sign allows the diagnosis of malaria. Most cases are accompanied by thrombocytopenia, a normal white count and a positive parasitaemia. Yet malaria must always be considered in cases of fever in the tropics. Since the symptoms can be quite diverse, a clinical diagnosis is unreliable and the diagnosis should be based on identification of the parasite. Microscopic confirmation of the diagnosis is often not possible in many regions and situations. It is of the greatest importance that other important diagnoses are ruled out before instituting a blind anti-malaria therapy. All too often fever is considered as malaria without considering alternative diagnoses. This tendency is reflected in the quote: “if you only have a hammer, you tend to see every problem as a nail” (Abraham Maslow).

The presence of parasites does not rule out an additional diagnosis: e.g. someone with fever may well have some malaria parasites in a thick smear, but this does not rule out meningitis or pyelonephritis. Chronic carriers are people who, although they have malaria parasites in their blood, have no symptoms of this. When such people develop another infection their

symptoms are often attributed to the malaria parasites in their blood, although these are not responsible. The absence of parasites in a single preparation does not rule out malaria but does make the diagnosis of *P. falciparum* highly improbable (if the microscopist searched carefully). Where there is any clinical suspicion it is best to repeat the test 12h later.

Microscopy

A **thick smear** concentrates the parasites 10 to 25 times. It is rather more difficult to interpret than a thin smear preparation and often does not permit species identification. A thick smear contains no intact red blood cells (haemolysis due to the distilled water used in the staining). If a thick smear is positive, a thin smear should be examined.

Parasitaemia

The parasitic density can also be roughly determined in a thick smear, by counting the number of parasites per 200 leukocytes and multiplying this by 30. It is assumed that on average there are 6000 leukocytes per µl blood and that there is one leukocyte per 500 red blood cells. For example: 5 parasites per leukocyte (1000 parasites for every 200 leukocytes) corresponds to a density of 30,000 parasites per µl. Roughly 30,000 parasites per µl corresponds to a parasitaemia of 1% (5 parasites per 500 RBC's): a moderately anaemic person.

If the thick smear is found to be negative in a reliable laboratory and if there is strong suspicion of malaria, the test is repeated every 12 hours for 48 hours. One great disadvantage of the thick smear method is that reliable technical expertise is needed which should be monitored (e.g. quality control). The argument that a lab technician has carried out the test for years and thus has plenty of experience is absolutely no guarantee of quality or reliability. The test also requires plenty of time if the parasitaemia is low, or before a negative result can be concluded.

A **thin blood film** has many advantages:

- it demonstrates the species present
- detection of mixed infections is possible

- distinguish asexual stages from gametocytes
- assesses parasitaemia (in % of infected red blood cells)
- can detect a new or unexpected parasite
- gives information on red cell morphology
- allows a white cell differential count
- inexpensive

Other points include: Sensitivity and specificity is operator dependent. In a good average lab, the sensitivity is good but limited to about 50 parasites per µL, this is somewhat better in a reference lab. Most routine laboratories cannot detect parasitaemia below 100 to 500 parasites per µL. DNA amplification techniques have better sensitivity and can give information when species is in doubt but this technique remains limited to reference laboratories (even in high resource settings).

If the parasite cannot be identified it is regarded as a *P. falciparum* as a safety precaution. Mixed infections do occur.

Antigen detection

Malaria rapid diagnostic tests (RDTs) based on lateral-flow immunochromatography are increasingly used in endemic and non-endemic settings. They are easy to use, provide results rapidly and require no specific training and equipment. Reported sensitivities vary between different RDT products but are generally good for *Plasmodium falciparum*, with rapid tests based on the recognition of *P. falciparum* antigen **histidine-rich protein-2** (PfHRP2) scoring slightly better than those which recognize *P. falciparum*–**lactate dehydrogenase** (LDH). Sensitivity is lower for *Plasmodium vivax* (66 – 88%) and usually poor for *Plasmodium ovale* (55 – 85%) and *Plasmodium malariae* (21 – 45%). Rapid diagnostic tests have some limitations. The test strips are susceptible to heat and humidity. A positive result can be obtained after correct treatment, when there are no more parasites visible in the thick blood smear. This is due to persistence of the PfHRP2 antigen (up to several weeks) after successful treatment. The pLDH based tests have the advantage of turning negative sooner after parasite clearance (several days). Occasionally there is cross-reactivity of *P. falciparum* with the non-falciparum test line and vice versa and rare false-positive reactions due to other infectious agents or immunological factors. False-negative results occur in the case of low

parasite densities, prozone effect (saturation of binding sites due hyperparasitaemia) or pfhrp2 gene deletions as observed in Pf strains from South America, but also in Mali, DRC and India. The latter two reasons for false negativity are only observed with HRP2-based RDTs. Finally when instructions are not followed (delayed reading, incorrect sample and buffer volumes, not recognizing invalid test results, disregarding faint test lines) errors in interpretation can occur. **Rapid diagnostic tests do not give information about parasite density.**

Depolarized light scatter

Automated cell counters, such as certain Cell-Dyn instruments, use 90° depolarized light scatter to distinguish eosinophils from other leukocytes. Eosinophils are normally the only leukocytes that depolarize light. Some automated haematology analyzers display an alert for possible malaria based on the presence of activated monocytes (Coulter Counter), hemozoin containing white blood cells (Cell-Dyn series) and an additional peak in the reticulocyte fraction (Cell-Dyn series). During malaria infection, the parasites consume haemoglobin and produce malariapigment, a form of polymerized haeme. This pigment, also known as hemozoin, is birefringent. When peripheral blood is analyzed by automated flow cytometry, the pigment will cause atypical depolarization of the laser beam that can be recognized in a scatterplot. Although diagnostic accuracy of these features is too low to exclusively rely on these flags for malaria diagnosis, such an alert is especially useful in situations where the initial clinical suspicion of malaria is low (non-endemic setting).

PCR

At present, in case of doubt, mixed infections, low parasitaemia, forensic questions, suspicion of zoonotic malaria, etc… PCR technology (e.g. multiplex real-time PCR) can give answers to several questions, but is in general slower than the traditional methods since such tests are not performed everyday even in larger centres. However, point-of-care PCR based techniques are being developed and their importance might grow in the future in countries contemplating malaria elimination, especially if this technique can combine detection of multiple infectious agents (multiplex-PCR). The future will learn whether they will have a place in diagnosis even in low-resource settings.

Serology

Serology can only be carried out in reference hospitals and is of no importance for the individual diagnosis in acute fever. The antibodies are positive from the tenth day therefore at the beginning of the attack they will be negative. The presence of antibodies only shows that there has been contact with the parasite. This does not mean that there is immunity. There will be high titers of antibodies in the tropical hyperreactive malaria splenomegaly. Malaria type IgG antibodies penetrate the placenta and will give the neonate temporary and partial protection against malaria during the first months of life. Antibodies after infection remain positive for a longer time.

Indirect aspects

Signs of haemolysis include yellow serum, dark urine while faeces have a normal colour, elevated indirect bilirubinaemia and low haptoglobin. Often there is thrombocytopenia. Sometimes there is malaria pigment in white blood cells (sign of severity).

Test therapy

In endemic regions fever, muscle pain or even generally feeling unwell are often attributed to “malaria”. An anti-malaria treatment is then instituted, without obtaining confirmation of the diagnosis or often even without considering alternative diseases. The argument given is that such a treatment can do no harm, that the diagnosis of malaria is always probable because the disease is common and that this is a good strategy for first-line care. Each of these arguments can be defended to a certain extent, but in this way often useless and sometimes expensive treatments with potential side effects are administered. In addition, not recognizing and treating other diseases (borreliosis, rickettsiosis, kidney infections, amoebic liver abscess, pneumonia, sepsis and so on) is a daily reality in many tropical regions. The over-diagnosis of malaria often leads to under-diagnosis of other treatable disorders. It is sometimes stated that fever which does not disappear after three days of adequate therapy, is not malaria. This may however not be completely true, in case of drug-resistant malaria (resistance R3, with no decrease in the parasite load during treatment) or co-infection with another pathogen (commonly sepsis).

In face of the increasing resistance to *P. falciparum* parasite and the need of more complex

and expensive treatment (ACT), WHO recommends since 2010 the diagnosis of malaria being parasite-based as often as possible either by microscopy or antigen-based RDTs. Ideally no malaria treatment should be provided without confirmation of the diagnosis.

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