Malaria in South Sudan 3: laboratory diagnosis

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Introduction

The previous article described the clinical features and diagnosis of malaria. However, for a definite diagnosis, the malaria parasite must be seen in a blood film. In this article we cover laboratory tests used to diagnose malaria (1, 2). These include:

- Microscopy including thick and thin blood films (best method for diagnosing malaria)
- Serodiagnosis such as the rapid diagnostic tests (RDTs) and Enzyme-Linked ImmunoSorbent Assay (ELISA).
- Other tests such as Polymerase Chain Reaction (PCR).

How to do the laboratory tests

What type of blood to collect

- Capillary blood (finger or heel prick)
- EDTA anti-coagulated venous blood
- Maternal placental blood.

When to collect the blood sample

- As soon as possible if malaria is suspected. However, it may be necessary to collect blood on several occasions to detect the parasite
- During peaks of fever
- Before the patient receives antimalarial drugs.

Note: Always ask the patient if he/she has taken any antimalarial drug.

Direct diagnosis of malaria

The blood film method for the laboratory diagnosis of malaria remains the gold standard in diagnosing malaria, i.e. blood film examination under the microscope. There are two kinds of blood films: thick and thin. The thick film is used for quick identification and quantification of parasites and the thin film is used for differentiation of parasite species.

You can prepare thick and thin blood films on separate slides or on the same slide. Common stains used are Field's stain, Giemsa stain and Leishman stain.

Thick blood film

This is suitable for the rapid detection of malaria parasites particularly when they are few.

Advantages:

- More sensitive by 30 times than thin films because:
  - * the blood is concentrated allowing a greater volume of blood to be examined and
  - * malaria parasites are concentrated as the RBCs are lysed
- Detects low parasitaemia.
- Can answer the question "Does the patient have malaria?" but only in experienced hands.

Disadvantages:

- Cannot differentiate between species of plasmodia.
- Parasites in the lysed cells are distorted
- Are more difficult to read so laboratories that have limited experience may prefer thin smears.

Thin blood film

This is the diagnostic tool most widely used to identify the parasite species.

Advantages:

- Confirms the plasmodium species
- Greatly assists in the identification of mixed

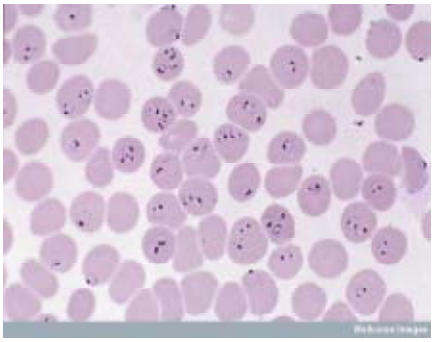

Figure 1: Ring stage P. falciparum. Note the multiplicity of rings within the red blood cells (© Wellcome Images. W0042190).

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infections

- Valuable in assessing whether a patient with falciparum malaria is responding to treatment in areas where drug resistance is suspected
- Provides an opportunity to investigate anaemia and white blood cell abnormalities in the absence of malaria parasites

Disadvantages:

- Less sensitive than a thick film especially where there is a low parasitaemia. This may delay diagnosis if, after a prolonged search, no parasites are found.

An explanation of the direct diagnosis

To establish the diagnosis of malaria, a blood film must be prepared from fresh blood obtained by pricking the finger or a heel (in case of baby/child).

The thin film is fixed in methanol before staining; the thick film is stained unfixed. Many hospitals in South Sudan have a Wright-Giemsa stain available, which is acceptable. However, Wright stain alone will not reliably show Plasmodium parasites. For the best results, the smear should be stained with a 3% Giemsa solution (pH of 7.2) for 30 - 45 minutes.

For rapid diagnosis, make thick and thin smears on separate slides. Air dry the thin film, fix it with methyl alcohol, and immediately stain it. If no parasites are found on the thin film, wait until the thick film is dry and examine it for organisms that might not have been detected on the thin film.

In P. falciparum infections, estimate the parasite density by counting the percentage of red blood cells infected - not the number of parasites - under an oil immersion lens on a thin film. Plasmodium parasites are always intracellular and they demonstrate, if stained correctly, blue cytoplasm with a red chromatin dot.

Common problems in reading malaria smears are caused by:

- platelets overlying a red blood cell
- concern about missing a positive slide
- misreading artefacts as parasites.
- poor staining
- partial haemolysis of red cells.

If smears are persistently negative, an alternative diagnosis should be considered.

A single negative blood film does not exclude malaria.

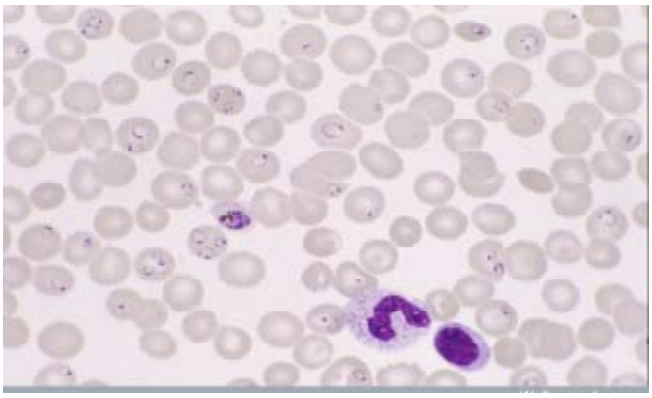

Figure 1: Thin film showing P. falciparum ring stage and a schizont in the centre of the slide.
(© Wellcome Images. W0042199)

A person suspected of having malaria but whose blood films do not show the presence of parasites should have blood films repeated approximately every 12--24 hours for 3 consecutive days. Only when all are negative, we can say that a febrile patient does not have malaria providing:

- we trust the competency of the laboratory technician and the facilities used and
- we can exclude other sources of errors (e.g. a patient received an anti malarial at home before coming to the health facility. This is a very common pitfall).

Figures 1, 2 and 3 show parts of the life cycle of the malaria parasite. See a full explanation in "Malaria in South Sudan: 1. Introduction and pathophysiology" in this issue of the journal.

Indirect diagnosis of malaria

Serodiagnosis of malaria

These are tests carried out by finding an antibody to the malarial parasite in the patient's serum. That is to say, a specific antigen (of a plasmodium species) is mixed with a patient's serum and, if reaction occurs, then it is positive but these kinds of tests have their own pros and cons. They are no use in the diagnosis of acute malaria because:

- They do not differentiate species
- They are tests that detect antibodies and an antibody can remain positive for years after a malarial infection.

Then when do we use these tests?

- To exclude malaria in a patient suffering from recurrent bouts of fever but presenting at the moment with no fever

- In surveys measuring the extent of population exposure to malaria. The commonest test is the Immunochromatographic Test (ICT). The ICT is the type of Rapid Diagnostic Test (RDT) available in South Sudan. The ELISA (Enzyme-Linked ImmunoSorbent Assay), another form of test used for detecting specific proteins of the plasmodia species, is not found in South Sudan.

Other new methods

- Quantitative Buffy Coat (QBC) is used when there is a low parasite count. A capillary tube is used to concentrate the blood and parasites are detected according to the specific gravity of the RBCs.
- Polymerase Chain Reaction (PCR) is used for detecting the parasitic DNA (mostly for detecting the resistant strains) and is a very sensitive method.

These modern methods are only useful in research studies and have never replaced the thick and thin blood films for routine clinical diagnosis of malaria.

Note: In South Sudan, only blood films and the antibody detecting test (ICT) are presently used.

Other laboratory investigations to accompany the blood film

- Haematological parameters (haemoglobin and haematocrit) to detect how far the RBCs are affected.
- Blood glucose – to detect how far the parasite has caused hypoglycaemia through stimulating glucogenolysis (which is a bad prognostic factor) and also before starting quinine which causes hypoglycaemia as a common side-effect.
- Coagulation studies (malaria affects the coagulation status)
- Screening for G6PD Deficiency (to avoid the antimalarials like primaquine that precipitates haemolysis)
- Renal function tests (malaria affects the kidneys either directly or by hypovolaemia caused by vomiting, diarrhoea and fever)
- Urinalysis (to detect haemolysis).

Laboratory diagnosis of malaria in children[3]

In all children suspected of severe malaria, check:

- thick blood film (and thin blood film if species identification required)
- haematocrit.

In children with altered consciousness and/or convulsions, check blood glucose.

Children with positive blood films and the following findings have severe malaria:

- severe anaemia (haematocrit <15%; haemoglobin <5 g/dl)
- hypoglycaemia (blood glucose <2.5 mmol/litre or <45 mg/dl).

In suspected cerebral malaria (i.e. children with unrousable coma for no obvious cause), perform a lumbar puncture to exclude bacterial meningitis - provided there are no contra-indications to lumbar puncture.

Contraindications to lumbar puncture

- Suspected increased intra cranial pressure especially if demonstrated by fundoscopy (i.e. papilloedema)
- Any suspected intracranial space occupying lesion e.g. brain abscess, tumour
- Coagulopathy
- Local infection at the lumbar puncturing site
- Midline shift in the Computer Tomography (not found in Southern Sudan).

Note: There are many contraindications divided into absolute and relative but these are the most common and important ones.

If you cannot exclude bacterial meningitis, give treatment for this also. If you suspect severe malaria on clinical findings and the blood film is negative, repeat the blood smear. Review the child clinically (history and examination) and review/consider the differential diagnosis, i.e. diseases causing febrile convulsions.

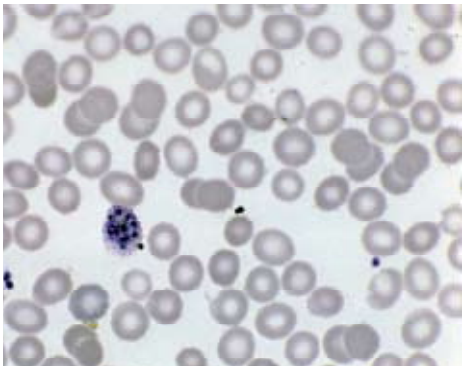

Figure 1: P. ovale schizont. Affected cell is oval, enlarged, irregular border and contain less than 12 merozoites
(© Wellcome Images. W0042150).

Laboratory prognostic indices

Laboratory indicators of poor prognosis in falciparum malaria include:

- Heavy parasitaemia i.e. when >5% of RBCs are parasitized
- Presence of mature trophozoites, malaria pigment and schizonts
- Peripheral leukocytosis of >12000/ml
- Low CSF lactate level
- Low antithrombin III levels
- Serum creatinine of >265umol/l
- Blood urea nitrogen >21.4 mmol/l
- Packed cell volume (PCV) of less than 20%
- Haemoglobin of less than 7.0g/dl
- Blood glucose of less than 2.2mmol/l
- Raised serum aminotransferases.

References

1. Ministry of Health, Government of Southern Sudan. Prevention and treatment guidelines for primary health care centres and hospitals. 2006; p98.
2. Gill GV & Beeching NJ. Lecture notes on Tropical Medicine 5th Edition. 2004; Chapter 9. p63.
3. WHO. Pocket book of hospital care for children - Guidelines for the management of common illnesses with limited resources. 2005. World Health Organisation, Geneva.

Further information

- Wellcome Trust. Malaria website at

  http://malaria.wellcome.ac.uk

- WHO. See the following sites: 'Diagnosis of malaria' at http://www.who.int/malaria/diagnosis_treatment/diagnosis/en/index.html and 'Malaria' at http://www.who.int/topics/malaria/en

Thanks to David Attwood for help in preparing this article and to the Wellcome Trust for allowing us to use their images.

Answers to Case History Quiz from Page 9.

A1. General condition is poor; the child is crying and looks as if he is suffering and in pain; a taught/tight membrane resembling parchment covers his skin. There is ectropion, eversion of lips, flattening of the nose and pus secretion from the eyes and base of the nose.

A2. Harlequin ichthyosis. This is characterised by the taught/tight membrane covering the entire body surface. The involvement of eyes (ectropion), mouth (fish-like deformity – eclabium) and distorted flat ears are typical features. Differential diagnosis: Collodion baby - which has a similar presentation of a taught membrane, but is less severe and lacks the additional facial features. The membrane in this condition typically breaks up and peels off in the 1st two weeks of life. Conditions associated with this include lamellar ichtyosis, ichthyosiform erythroderma and Netherton's syndrome.

A3. This is a clinical diagnosis. Questions that can help in making the diagnosis are: a) Is the couple consanguineous (blood relatives)? b) Does the couple have another child with ichthyosis? c) Does the family have a history of severe skin disorders?

A4. It is an autosomal recessive disease, transmitted by both parents. In this case they are direct/first cousins.

A5. Give: a) Systemic antibiotics. The use of prophylactic antibiotics is debatable, but probably indicated if the baby cannot be nursed in a sterile environment. b) IV fluid. This is very important as affected infants are often unable to feed.c) Systemic retinoids. Acitretin 1mg/kg/day and isotretinoin 0.5mg/kg/day both enhance/improve survival and reduce morbidity. Water is lost through the skin. So bathe the baby twice a day and use sodium chloride compresses followed by bland emollients to soften hard skin. Do not use salicylic acid preparations. Use eye lubricant ointments rather than eye drops.

A6. No, this child seems very seriously affected. Possible causes of death are sepsis, dehydration and malnutrition.

Thanks to Chris Bower for helping to provide answers for the quiz.

SSMJ is proud to support the continuing development of the health care system in South Sudan as the country moves towards Independence. Please share with us your experiences during this period, particularly those related to the challenges caused by the large number of 'returnees'. Email admin@southernsudanmedicaljournal.com