

3: VL AND LABORATORY DIAGNOSIS

3.1 How is microscopy performed for parasitological diagnosis?

Smears of aspirates or biopsies (see section 2) arriving from public health centres or family doctors should all ideally be examined on the day of arrival. If slides have to be kept over night they should be stored dry in closed containers at room temperature.

If smears are prepared from patients at the laboratory they should be dried rapidly (e.g. by rubbing the back of the slide with a finger to slightly warm the glass and drive off moisture) and then fixed immediately with absolute (100 %) methanol (methyl alcohol) for one minute. The methanol must be stored in tightly closed bottles to prevent absorption of water.

Stain the films with Giemsa as described in Appendix 5.

Examine the Giemsa-stained smears using a 100X oil immersion objective. *Leishmania* amastigotes are very small round or oval organisms about 3 μm x 5 μm found inside or outside phagocytic cells (macrophages). Each amastigote contains a red-mauve nucleus, a smaller more deeply staining red-mauve kinetoplast, and pale blue cytoplasm. The particular diagnostic feature to look for in VL is the presence of nucleus and kinetoplast in these organisms (Slides 25, 26 and 27).

In about 50 % of patients that are severely immunosuppressed, such as HIV co-infected individuals, *Leishmania* amastigotes may be found in thin films or thick smears of peripheral blood.

The sensitivity of parasitological examination can often be increased by aseptically placing aspirate or biopsy material into culture medium and then examining the medium after several days. [The preparation and inoculation of culture media is described in Appendix 4.] This has the disadvantage that an immediate diagnosis cannot be provided. Also, if laboratory staff are not skilled in the preparation of the medium and inoculation of the medium with aspirate and biopsy material, cultures are very prone to contamination with bacteria or fungi. *Leishmania* usually grow in culture as free-swimming, flagellated promastigotes, although they may also divide as clumps of amastigotes (Appendix 4, Slide 28).

3.2 What haematological signs may be associated with VL?

Haematological tests in the laboratory that are useful for detecting signs of VL are the haematocrit to determine packed cell volume (PCV), determination of haemoglobin (Hb),

white cell count (Appendix 5) and determination of total serum protein.

These tests may indicate:

Anaemia, especially in severe cases of VL.

Reduced white cell counts (leukopenia) with total white cell counts down to $2.0 \times 10^9/l$.

An increase in serum total protein (Slide 29).

There may also be a very high erythrocyte sedimentation rate (ESR) and reduced numbers of platelets (thrombocytopenia) leading to a prolonged blood-clotting time.

3.3 **What serological tests are useful for diagnosis of VL?**

Available serological tests are the formol gel test (aldehyde test) in tubes or on slides (useful in the absence of any other), the direct agglutination test (DAT), the indirect immunofluorescence test (IFAT) and the enzyme-linked immunosorbent assay (ELISA or Dot-ELISA). These tests are described fully in Appendices 6 (formol gel test), 7 (DAT test), 8 (IFAT) and 9 (ELISA) (Slides 30, 31, 32 and 33).

The formol gel test and DAT (given a reliable standardised source of DAT antigen) can be performed outside laboratories in primary health care centres. The IFAT and ELISA tests require laboratory facilities and more sophisticated equipment, although rapid ELISA or dipstick tests are being developed for use in primary health care centres or in the home.

3.4 **What diagnostic methods should be used to follow-up patients who have been treated?**

In treated patients who show no clinical and/or haematological improvement or who have a clinical relapse, parasitological diagnostic tests should be repeated to determine if *Leishmania* are still present. Parasitologically positive patients will require re-treatment, possibly with a modified course of treatment or a second line drug or combination of drugs (see section 4).

For patients who have shown good clinical improvement a positive leishmanin skin test (Montenegro; Appendix 10, slide 34) provides confirmation of cure.

3.5 **What minimum or special equipment and services are required for laboratory diagnosis?**

For microscopical examination of stained smears: microscope with (50X) or 100X oil immersion objective, microscope slides, staining trays, syringes (10 or 20 ml), needles, sodium chloride, disodium hydrogen phosphate, Giemsa stain.

Other equipment and services for the parasitological, serological and haematological tests are described in the Appendices (4 - 10).