

2: VL AND THE PHYSICIAN
(Clinical diagnosis and referral)

2.1 **What features are used to make a differential clinical diagnosis?**

As an example these are the clinical features recorded for patients with VL in the Sudan, Brazil and India.

	Sudan	Brazil	India
Fever	95%	95%	99%
Splenomegaly	95%	99%	98%
Uncomfortable spleen	85%	50%	50%
Weight loss (wasting)	80%	98%	87%
Anaemia	75%	98%	96%
Lymph node enlargement	75%	30%	90%
Loss of appetite	70%	20%	30%
Cough	75%	40%	50%
Hepatomegaly	60%	90%	98%
Epistaxis (nosebleed)	50%	30%	10%
Diarrhoea	40%	60%	50%
Vomiting	15%	infrequent	infrequent
Jaundice	5%	10%	
Oedema	5%	40%	

These clinical features are common to all endemic areas but some such as lymph node enlargement are far less frequent outside the Sudan and India.

The incubation period for VL is typically 2-6 months but may be shorter or much longer. The onset may be gradual or acute.

In many endemic areas malaria is the most common infection that can have a similar clinical presentation. VL should be considered in patients with a prolonged irregular fever, accompanied by other suggestive symptoms (above) when there is no response to anti-malarials and no malaria parasites can be found by repeated examination of blood films.

In all countries differential diagnosis must also consider typhoid (enteric fever), tuberculosis, AIDS, brucellosis, chronic hepatitis, cirrhosis, lymphomas and leukaemia. Massive splenomegaly may be a feature of VL, portal hypertension (due to cirrhosis and schistosomiasis) and malaria (due to hyperactive malarial splenomegaly).

Laboratory diagnosis, if available, to detect leukopenia (85 % of patients in the Sudan), thrombocytopenia (75 % of patients in the Sudan) and positive serum antibody (95 % of patients who do not have AIDS) is valuable for confirming clinical diagnosis (see section 3).

2.2 **What atypical clinical presentations might occur?**

Occasionally the clinical presentation is atypical, without splenomegaly but with fever, wasting, diarrhoea, cough, or combinations of these. In West Bengal, India, generalized lymph node enlargement without visceral involvement was reported.

Simple cutaneous lesions (leishmanioma) may occasionally precede, accompany or follow VL. PKDL may occur before VL in rare cases, or in Africa begin during treatment.

Infections due to *Leishmania infantum/Leishmania chagasi* (Appendix 2) are apparently often asymptomatic. Most individuals who have evidence of exposure to *Leishmania*, with a positive leishmanin skin test or positive serology (typically 3 - 30 % of the population in endemic areas) do not recall having a clinical illness. Infection with *Leishmania donovani* (Appendix 2) is thought to be more frequently clinically apparent than infection with *L. infantum/L. chagasi*, but subclinical VL is always more common than clinical VL.

2.3 **When is clinical diagnosis a basis for initiating drug treatment?**

When laboratory facilities are not available, as clinical (symptomatic) VL (see 2.1 and 2.2 above) is not benign or self-limiting and it is usually fatal if untreated, appropriate treatment should be given.

2.4 **What complications and secondary infections might occur in VL?**

Secondary infections are common, and include pneumonia, bronchial infections, tuberculosis, malaria, diarrhoea or dysentery, viral infections, bacterial skin infections, otitis media (inflammation of the middle ear), and cancrum oris (mouth lesions).

Thrombocytopenia may cause epistaxis (nosebleed), or bleeding from other sites, and this may precede death.

Leishmania enteritis may be a cause of diarrhoea and malabsorption and pulmonary involvement may mimic pneumonia.

Death is mainly due to secondary infection, or haemorrhage.

2.5 **What immediate clinical responses are appropriate?**

- (a) A patient should ideally be referred to a centre where diagnostic facilities, drugs and expertise in the treatment of VL are available. If lack of resources or other logistic problem prevent this, treatment should be given even in remote areas as soon as possible (see section 4).
- (b) Coexisting malaria, anaemia, bacterial infections and tuberculosis should all be treated simultaneously.
- (c) The patient should be provided with adequate food during treatment.
- (d) The physician should make an enquiry about other cases of VL in the family and village, and notify the relevant health authorities about the existence of VL.

2.6 **How can samples be collected for serological diagnosis?**

- (a) *For the direct agglutination test (DAT):*

Puncture the patient's finger with a lancet (after cleaning the skin thoroughly with a 70% alcohol swab), turn the finger downwards and collect two drops of blood (each making a separate blood spot of 1 cm diameter) on to Whatman No. 3 filter paper. Make sure the finger never touches the filter paper during the operation.

Label the filter paper with the patient's name, code number and date (Slide 21).

Allow the blood spots to dry for few hours at room temperature and then store them dry singly in a plastic envelope or sealed container, or with clean dry paper

separating filter paper sheets.

The filter papers may be stored at room temperature for one week, for months in a 4°C fridge, or for years in a -20°C freezer. One blood spot should be used for diagnosis and one saved for future reference or repeat testing.

The blood spot collection method is also sometimes used for other serological tests, such as the indirect immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) (see (b) below).

Serum ((b) below) can also be used for the DAT and may give more accurate results.

- (b) *For the indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA):*

Take about 1 to 2 ml of blood from the vein with a sterile syringe and needle into a sterile container without anticoagulant. Allow the blood to clot, take off the serum, discard the red cells, and store the serum at 4°C in a fridge, or at -20°C if longer storage is needed.

- (c) *For the formol-gel slide test:*

Serum can also be used for this test. It is not specific for VL but detects hyperglobulinaemia, which is often associated with VL.

2.7 **How can samples be collected for parasitological diagnosis?**

- (a) *Bone marrow aspirate:*

Under local anaesthetic, aspirate a sample (up to 1 ml) of bone marrow from the iliac crest or the sternum using a sterile bone marrow aspirate needle and a 10 ml syringe. Immediately make thin smears of the aspirate on at least three glass microscope slides. Allow smears to air dry (or dry rapidly by rubbing the back of the slide with a finger to slightly warm the glass and drive off moisture), fix with 100 % methanol (methyl alcohol), dry, label and store protected from insects until staining. Stain with Giemsa or May-Grünwald Giemsa (section 3). Examine at least 1000 fields per slide under oil immersion (100X magnification) preferably around the edges of the preparation.

If culture medium is available carefully inoculate culture tubes with one or two drops of the aspirate, using aseptic precautions (swab the culture tube rubber cap with 70 % alcohol and inoculate through the cap) to avoid contaminating the

medium. The medium should be NNN (Slide 22), blood agar, or sloppy Evan's medium (Appendix 4). Keep culture tubes at room temperature (25°C optimal). In very hot countries steps must be taken to prevent the cultures from overheating, e.g. cover with a damp towel or place near a ventilator to keep the temperature down.

(b) *Lymph node aspirate (Slide 23):*

Inguinal and epitrochlear lymph nodes are most convenient. Grasp the lymph node between thumb and fingers, introduce a 21 gauge needle attached to a 5 ml syringe into the lymph node, milk (gently press the lymph node several times) or move the needle backwards and forwards several times, and remove it. Make smears (and cultures if available, and if there is sufficient material) as in (a) above.

(c) *Splenic aspirate (Slide 24):*

THIS PROCEDURE MUST NOT BE PERFORMED WITHOUT TRAINING AND EXPERIENCE: IT MAY LEAD TO FATAL HAEMORRHAGE IF DONE INCORRECTLY.

First ensure the patient is not at risk of bleeding, and that facilities are on hand to deal with bleeding. The platelet count should be greater than 40,000 and prothrombin time greater than 50%. (See WHO technical report series 793 annex 4 for a full description of requirements.) In brief, fix the spleen between thumb and forefinger of the open hand. Introduce a 21 gauge needle attached to a 5 ml syringe just through the skin, and draw back the plunger to the 1ml mark to apply suction. Maintaining suction throughout and with a very rapid in and out movement, push the needle into the spleen to the full needle depth (3 cm) and then withdraw it immediately and completely. The entry and exit trajectory of the needle must remain the same and should be perpendicular to the spleen.

Notes:

Material from splenic and lymph node aspirates will be very scanty, and the whole specimen may be needed to prepare the smear. The contents of the syringe may have to be briskly squirted out of the syringe onto the slide in order to obtain an adequate specimen. The syringe and needle may then be rinsed in culture medium for inoculation of culture tubes.

If it is necessary to send diagnostic samples (e.g. live cultures) to a reference centre through the mail, local legal requirements must be checked and followed.

2.8 **What minimum or special equipment and services are required for clinical**

diagnosis and collection of samples for laboratory diagnoses?

An examination area; clinical thermometer; microscope; microscope slides; Giemsa stain; bone marrow aspiration needles; disposable syringes (5 ml and 10 ml); disposable needles; local anaesthetic; blood lancets; filter paper; plastic bags or boxes; plastic screw-top blood and serum containers; pasteur pipettes; access to serology locally or by referral of samples (access to treatment for all cases detected - see section 4).

2.9 To whom should cases of VL be reported?

A diagnosis of VL should be followed by notification to the local, regional and national health authorities, irrespective of whether the case of VL is parasitologically confirmed or not. Details should include the patient's name, age, sex, locality (address), the results of tests if known, whether the patient has travelled to an endemic area (where, when and for how long), whether the patient is immunosuppressed, and whether the patient is part of a cluster or outbreak.

2.10 What other action should the physician take?

The physician should try to determine whether other cases of VL exist in the same family or/and in neighbouring households, by active case finding, and should communicate with authorities in neighbouring areas, to discuss the extent of the problem. In the case of VL with a (canine) reservoir (zoonotic VL), the physician should assist veterinary and environmental health teams.

In all instances the physician should try to ensure that patients diagnosed as having VL receive a full course of treatment at the right dose and without any interruption to prevent drug-resistance, relapse, and PKDL (see section 4).

Case histories of VL (immunocompetent)**Case History - VL - 1**

A girl, aged 13 years, from a VL endemic area in Kenya was admitted to a district hospital complaining of fever for three months, left sided abdominal pain, tiredness and loss of weight.

Examination revealed pallor and severe emaciation, 12 cm palpable splenomegaly, 5 cm palpable liver and generalized lymphadenopathy.

Investigations showed: Hb 6.6 gm/dl WBC $2 \times 10^9/l$, and a negative blood film for malaria. The DAT titer was 64,000. A lymph node aspirate was positive for *Leishmania*.

The patient received pentavalent antimony, 20 mg/kg body weight daily for 30 days, multivitamins and iron. The fever subsided and the spleen size regressed by 2 cm in the first

week of treatment. The patient started to gain weight and her general condition improved steadily. She was discharged after completion of treatment in good condition. The spleen remained just palpable for 12 months, but there were no symptoms of relapse.

Case history - VL - 2

A 10 year old boy from Sudan was admitted to hospital complaining of fever, diarrhoea and epistaxis for two weeks.

On examination: the patient was pale and wasted. The spleen was just palpable but the liver and lymph nodes were not enlarged. Investigations showed: pancytopenia, a negative blood film for malaria. The DAT titer: 102,400. Bone marrow smear was positive for *Leishmania*. The patient was treated with pentavalent antimony (20 mg/kg body weight) daily for 30 days, multivitamins and iron. After one week of treatment the fever subsided and the patient was feeling better with increasing appetite. The patient was discharged in good condition after 30 days of treatment. He returned for follow-up at 3 and 6 months and continued to be in good health.