

Leishmaniasis in AIDS patients: results of leukocytoconcentration, a fast biological method of diagnosis

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Leukocytoconcentration is an easy, fast and inexpensive technique for the diagnosis of leishmaniasis from peripheral blood. The technique involves concentration of blood parasites on a small surface of a microscope slide while the red blood cells are removed by lysis. The results, compared with those of other methods (examination of cultures of blood samples and bone marrow smears), were very good and accurate. All but one of our cases of leishmaniasis were patients with HIV co-infection. Leukocytoconcentration facilitates follow-up of cases and fast detection of any relapse.

Ideally the biological diagnosis of an infectious disease is based on isolation and identification of the pathogenic agent in the host tissue and establishment of cultures after direct examination under a microscope. This procedure allows both accurate diagnosis and an epidemiological survey of the disease.

However, when leishmaniasis occurs in an AIDS patient or any other immunocompromised patient, this procedure is often unsatisfactory for several reasons: the samples are difficult to collect, there may be few parasites, and their growth is slow or impeded by other pathogenic agents. The clinical features, when they are not specific, can be attributed to an etiology other than leishmaniasis, and this does not encourage the repetition of laboratory tests (1, 2).

This report describes an easy, fast and inexpensive technique for the diagnosis of leishmaniasis from peripheral blood, and compares the results with those of other techniques.

Materials and methods

The study, which was carried out during the two years 1993–94, involved 66 patients who were living in Paris or its suburbs but had been infected by leishmaniasis in the Mediterranean area. Most of

them were co-infected with the human immunodeficiency virus (HIV).

Direct blood sample examinations were carried out by the Parasitology Laboratory of the Avicenne Hospital, using a new technique called leukocytoconcentration (LCC) in which the blood parasites are concentrated on a very small surface of a microscope slide, the red blood cells and platelets being removed by lysis. This technique is derived from a single leukoconcentration (3) and one previously used for malaria parasite detection (4), with modifications as described below.

The LCC technique

- (1) Collect a 5 ml blood sample in citrate.
- (2) Take a blood volume correlated to the number of leukocytes, according to the following formula: volume (in ml) = 1250/leukocytes per ml.
- (3) Fill a 10 or 15 ml centrifuge tube with this volume and complete with nine volumes of haemolysis solution (0.4% saponin (UCB, Brussels, Belgium), 2.5% formalin, and 0.5% glycerol, made up to 100 ml with physiological saline, filtered twice and stored at room temperature). Using a pipette, aspirate and expell the solution 3 or 4 times over 15 minutes (to mix it properly) before centrifugation.
- (4) Centrifuge at 840g for 10 minutes (centrifuge: Jouan, Saint-Herblain, France), and pour off the supernatant fluid carefully.
- (5) Add 100 µl of 0.9% saline and dissociate the pellet by homogenization.
- (6) Cyto-centrifuge at 450g for 10 minutes (cyto-centrifuge: Miles Laboratory Inc., IN, USA), the

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leukocyte nuclei and leishmanias spread over the limited surface of the slide.

(7) Dry the slides and stain with May-Grünwald Giemsa.

(8) Examine under a microscope with a $\times 100$ objective after coating the spot with oil.

Parasites from the same blood samples were systematically isolated by the Parasitology Laboratory of the Henri Mondor Hospital using the standard culture technique in Novy, McNeal, Nicolle (NNN) medium overlaid with Schneider's medium and fetal calf serum. Isolation allowed identification of the stocks by isoenzyme analysis. The usual bone marrow, skin and mucosal biopsies were also explored for these parasites, when the clinicians could obtain the necessary samples.

Results and discussion

A total of 116 leukocytoconcentrations, 12 bone marrow punctures and 128 cultures on NNN medium were analysed from the 66 patients in the study, of whom 12 were cases of leishmaniasis.

Blood LCC versus bone marrow puncture (Table 1). Only 12 bone marrow smears were available, because puncture is a traumatic procedure and could not be requested in cases after the diagnosis had already been established. No statistical significance can therefore be deduced from these smears. Nevertheless, four positive smears correlated with a positive LCC; in two cases, bone marrow punctures did not allow a diagnosis whereas LCC showed the presence of leishmanias in the blood. Bone marrow punctures cannot easily be repeated and need specialized personnel, which could delay the diagnosis and a medical decision. In contrast, venous blood puncture by a nurse is easy and well tolerated.

Parasitaemia occurs with *Leishmania donovani* (1) and is unusual with *L. infantum*; but recently in AIDS patients parasites have been found in blood (5, 6), and the LCC technique enhances the possibility of immediate direct diagnosis.

Table 1: Results of diagnosis by leukocytoconcentration (LCC) and bone marrow smears (BMS)

Positive (+) or negative (-)	n
LCC+, BMS+	4
LCC+, BMS-	2
LCC-, BMS+	0
LCC-, BMS-	6

Table 2: Results of diagnosis of leishmaniasis by leukocytoconcentration (LCC) and cultures of blood specimens (C)

Positive (+) or negative (-)	No. in 1st sample	No. in follow-up	Total
LCC+, C+	10	23	33
LCC+, C-	2	3	5
LCC-, C+	0	12	12
LCC-, C-	54	12	66
Total	66	50	116

Blood LCC versus blood cultures (Table 2). The cultures are used as control tests. In the present series, there was a strong correlation between the results of LCC and cultures from the same blood samples ($P < 0.001$), and despite the limited number of samples, the possibility of immediate diagnosis thus obtained is essential for clinicians. Our results must of course be completed by the isolation of the parasites, because their identification is of great interest from both epidemiological and medical viewpoints. Well supplemented NNN medium allows the isolation for identification of the parasite, but the growth of parasites from blood is slightly slower than that of parasites isolated from bone marrow punctures.

The LCC method is simple and inexpensive and gives very good results, whereas the method of Medrano et al. (7) only permitted the detection of half the positive peripheral blood smears in patients co-infected by leishmaniasis and AIDS. Sophisticated techniques such as the polymerase chain reaction or antigen detection have been studied and their results seem to be in good agreement with those of other techniques. However, they are expensive and cannot be performed routinely by all laboratories. Besides, they do not allow the isolation of parasites, which is important for sensitivity tests and species identification.

The presence and, above all, the abundance of parasites in the blood are certainly a help for diagnosis, confirming the position of humans in the epidemiological scheme. Formerly, humans were not considered a reservoir host of *L. infantum*. However, in endemic areas now, an infected subject, especially when co-infected with AIDS, could be a reservoir for sandflies, as suggested by Molina, who infected sandflies using the blood of HIV patients (8). The position of humans in the *Leishmania* life-cycle is sometimes important; thus, in some foci of *L. donovani*, humans are an excellent reservoir of infection (9).

Conclusion

The unreliability of the usual techniques for the diagnosis of visceral leishmaniasis, particularly in immunocompromised patients, led to the search for new methods such as the polymerase chain reaction or circulatory antigen detection. While these are effective, they are expensive and cannot yet be used routinely.

Leukocytoconcentration, involving isolation of parasites from the blood, is a process which is both reliable and well tolerated by patients who do not always comply with the need for follow-up examinations after therapy. It is capable of making an important contribution to diagnosis; the financial aspect must also be considered and we suggest a multi-centre evaluation to determine the importance of this technique in the search for parasites from peripheral blood.

Résumé

La leucocytoconcentration, une méthode biologique rapide de diagnostic de la leishmaniose chez les malades du Sida.

Les auteurs décrivent la leucocytoconcentration (LCC), une technique rapide, sensible et de réalisation facile pour le diagnostic de certitude de la leishmaniose viscérale. La LCC est réalisée à partir du sang périphérique des patients. Elle concentre les parasites sur la plus petite surface possible d'une lame porte-objet. Ses résultats sont contrôlés par ceux de la culture sur milieu NNN et comparés à ceux obtenus par ponction de moelle

osseuse. Tous les malades diagnostiqués, sauf un, sont coinfectés par le VIH.

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