Malaysian J Pathol 1989; 11:1-5

RECENT ADVANCES IN THE LABORATORY DIAGNOSIS OF FILARIASIS

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Summary

The specific laboratory diagnosis of filariasis depends either on the demonstration of circulating microfilaria in the peripheral blood or various stages of the parasite in tissue sections. Various morphological characteristics of the parasite will normally assist in its identification to the genus and specific level. Concentration techniques, especially those using the polycarbonate membrane filtration of a ml or more of heparinised blood, can detect the parasite in those with very low microfilaria counts. Serological techniques using the indirect fluorescent assay with microfilaria and adult worm antigens can assist in confirming a clinical suspicion of filaria infection when the parasite is not demonstrable. The development and use of specific monoclonal antibodies for fhe detection of circulating antigens in the enzyme-linked immunosorbent assay will probably increase the specificity of the assay. Presently available specific DNA probes for filariasis are probably not as useful for patient diagnosis as they are for epidemiological purposes.

Key words: Filariasis, diagnosis.

INTRODUCTION

The laboratory diagnosis of lymphatic filariasis is based mainly on parasitological, histopathological and immunological approaches. While there is no significant advance in these techniques, some refinements in their use and the interpretation of results have occurred and will be discussed in this review. Furthermore, some recent applications of molecular biology and biotechnology in filariasis which will have some impact on future diagnostic tests will be discussed.

PARASITOLOGICAL TESTS

The demonstration of microfilaria in the peripheral blood, urine or in body fluids like hydrocoele fluid remains a useful and specific test for the diagnosis of a current fiiarial infection. However, the thick blood smear technique normally employed to detect microfilaria is insensitive and as both *Wuchereria bancrofti* and *Brugia malayi* microfilaria peak in the peripheral circulation at night, a nocturnal specimen is normally needed for diagnosis to increase the chance of detecting the infection.

The administration of 50 mg and 100 mg of diethylcarbamazine citrate (DEC) to those below 12 years old and those 12 years and above respectively, 30 – 45 minutes before blood examination, obviates the need for nocturnal samples of blood. This technique for the day-time examination of blood for microfilaria has been shown to be effective even for the diagnosis of periodic B. *malayi* infection.[1] The giemsa-stained 60 ul thick blood smear for the demonstration of microfilaraemia allows the quantification and specific identification of the microfilariae. It even allows for the strain identification of the parasite as the periodic form of B. *malayi* can easily be differentiated from that of the subperiodic form as more than 50% of the microfilariae of the former are normally exsheathed when the film is dried at room temperature, while more than 90% of the latter strain are normally sheathed. This strain differentiation is important as the subperiodic, unlike the periodic strain, is a zoonotic parasite.

Various modifications to increase the sensitivity for the direct demonstration of circulating microfilaria have been developed, including the Knott's concentration and the membrane filtration techniques. Essentially, these are concentration techniques whereby a ml or more of heparinised blood can be processed and microfilariae, if present, are concentrated for easy detection. The former technique is simple and economical as it only involves the addition of a ml of blood to 10 ml of a 2% formalin solution, thorough mixing, centrifugation and examination of the sediment prepared as a methlylene-blue or giemsa-stained smear on a glass slide. However, the technique can be tedious and requires the use of a centrifuge. The filtration of a ml or more of heparinised blood, mixed with 10 ml of normal saline through a polycarbonate membrane,

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25 mm in diameter, with 5 um pore size, effectively concentrates microfdariae onto the membrane, which can then be placed on a glass slide, dried, fixed with absolute methanol, stained with giemsa and examined directly under the microscope (Fig. 1). This is a rapid, sensitive, specific and easy technique to carry out. These two concentration techniques require venepuncture to obtain the 1 ml of blood needed. However, the membrane filters are expensive, costing about MS1.70 each. These two techniques should be used to detect low microfilaraemic states as in tropical pulmonary eosinophilia and to monitor the effectiveness of chemotherapy, where microfilaria counts may become very low.

Sometimes larvae or adult worms may be recovered from conjunctival cysts,[2-6] the vitreous of the eye[7] or from other sites. In such instances, identification of the parasite to the generic or specific level, based on well known morphological characteristics and measurements can normally be made.

HISTOPATHOLOGICAL DIAGNOSIS

Larvae or adult filaria parasite sections are sometimes encountered during histological examination of pathological specimens. In such instances, the parasite must be differentiated from other tissue dwelling or migrating parasites such as *Trichinella, Toxocara, Strongyloides,* etc. However, a filaria parasite can normally be correctly identified to the generic and sometimes specific level, based on the size, cuticular and internal morphology and the microfilariae in the uteri. It is important to make serial sections of the specimen to help in the correct identification of the parasite (Figs. 2 & 3).

Filaria parasites encountered in tissue sections in this country have been identified as *Brugia, Wuchereria* or, *rarely, Dirofilaria.* The adult male of B. *malayi* has a mean width of 75 (range 70–80) um while the female measures 148 (range 70–145) um. Corresponding measurements for B. *pahangi* are similar.[8] The rural *W. bancrofti* adult male has a mean width of 108 (range 90–120) um while that of the female is 174 (range 160–188) um.[9] *Dirofilaria* worms are generally much larger than the above parasites, the adult male and female *D. immitis* having widths of 600–900 um and 1,100 um respectively; widths of adult male and female *D. repens* are less, these being 370–450 um and 450–650 um respectively.[10] The male and female of *D. magnilarvatum,* the other common *Dirofilaria* in the country, have widths of about 336 um and 422 um respectively.[11]

There are few studies on the measurements of larval stages of the above filaria parasites. The mean widths of one and two weeks'old

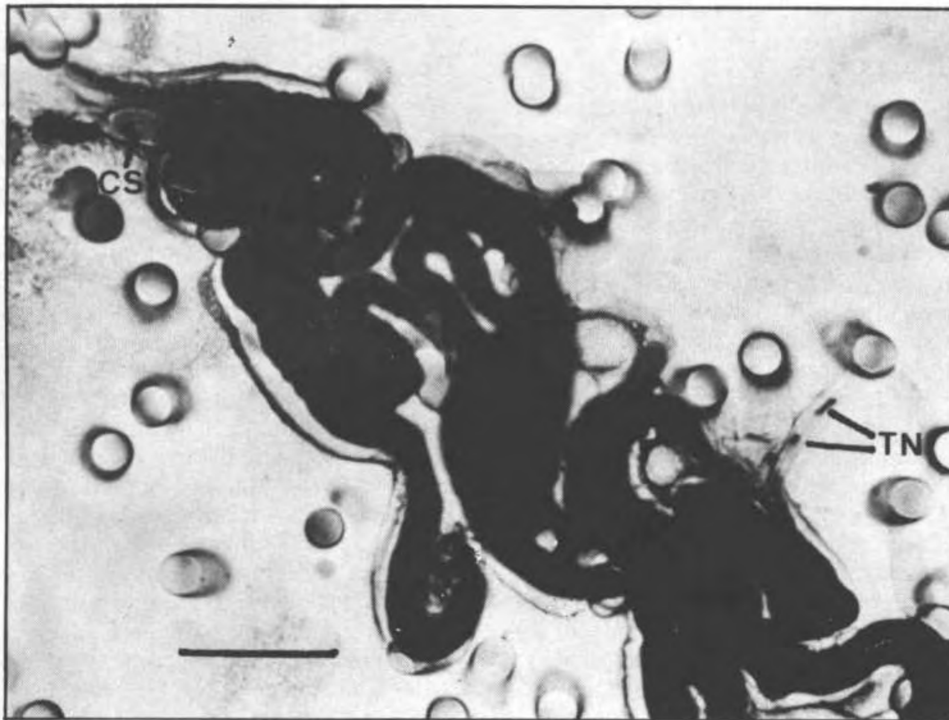

FIG. 1. Nucleopore (R) membrane concentration of blood for microfilaria. Pore size, 5 um. Five *Brugia malayi* microfilariae seen here, with typical cephali space (CS) and terminal nuclei (TN). Scale = 20 um.

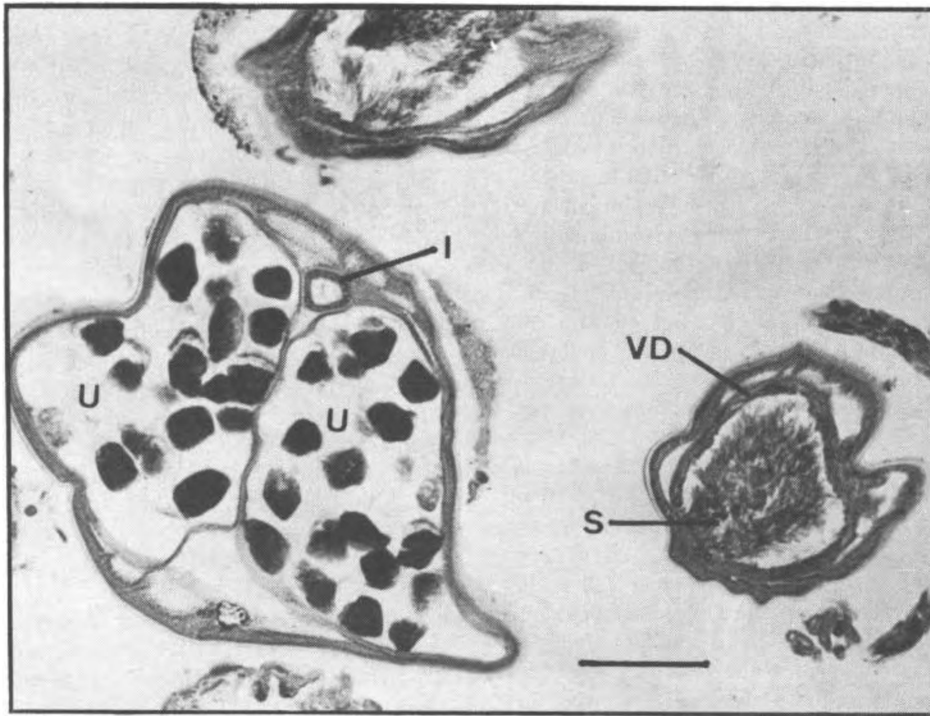

FIG. 2. Cross sections of *Brugia* adult worms in a subcutaneous cyst in the neck region of patient. Section of female worm on the left side, showing two uteri (U) with ova, filling the body cavity of worm, and intestine (I). Male .worm at right showing vas deferens (VD) with spermatozoa (S). Note absence of inflammatory cells around living worms. Scale = 40 um

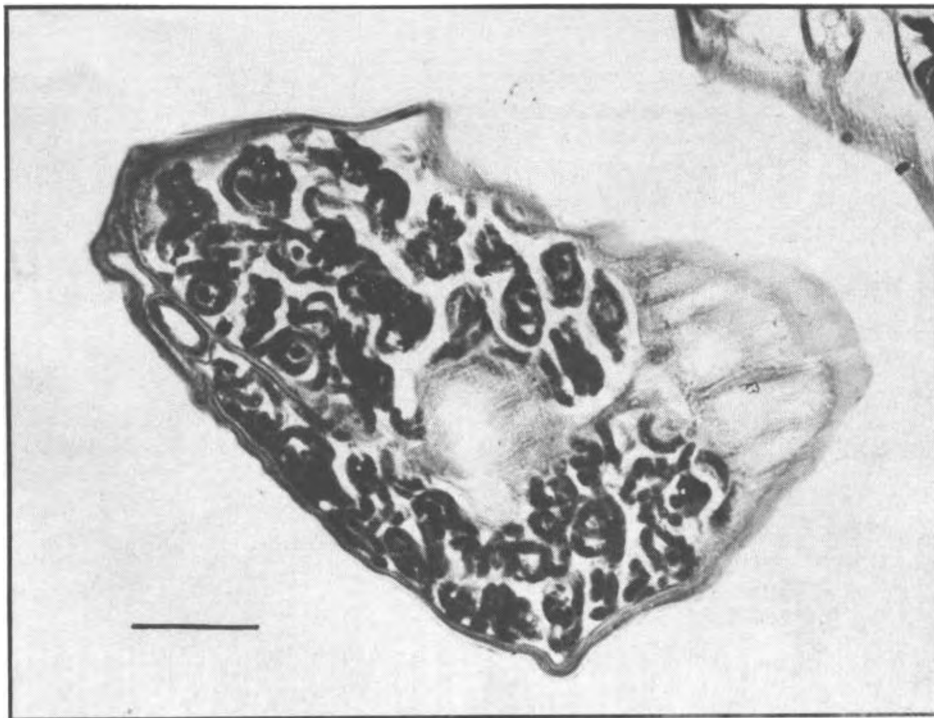

FIG. 3. Oblique section of female gravid *Brugia* with microfilariae in uteri. Scale = 40 um

B. malayi larvae measure 60.7 ± 2.6 and 69.2 ± 1.8 um respectively.[12]

Living microfilaria, larva and adult filaria parasites normally do not provoke any inflammatory response unless they are affected by antifilarial drugs or when their evasion of host immune responses fail. In such instances, the histological changes are very similar, the dead or dying parasite being surrounded by eosinophilic material, eosinophils, chronic inflammatory cells, including epitheloid and giant cells (Fig. 4). In the affected afferent lymphatics, the parasites may be enclosed in a thrombus of granulomatous reaction, together with perilymphangitis, biocking the lumen.

In occult filariasis, which is defined as filarial infection where there may be enlargement of the lymph nodes, hypereosinophilia, pulmonary symptoms but no circulating microfilaria, there is pronounced lymph node follicular hyperplasia and microfilaria or their remnants surrounded by hyaline materials known as Meyers-Kouwenaar bodies are seen within pools of eosinophils. Epitheloid granulomata with foreign body giant cells can be found in various organs, including the lungs, liver and spleen. Meyers-Kouwenaar bodies may be seen in these granulomata.

IMMUNOLOGICAL DIAGNOSIS

A number of serological tests are used to confirm the filarial aetiology of clinical conditions such as lymphoedema and elephantiasis of the limbs, breast, scrotum or occult filariasis where microfilaria is not demonstrable. These tests are also necessary to help in the differential diagnosis of hypereosinophilia with pulmonary signs and symptoms. Commonly available serological tests include the indirect fluorescent antibody (IFA) assay, using B. *malayi* intact or sonicated microfilaria, and frozen sections of adult worms. Titres of 1:40 and above with microfilaria antigen or 1:8 and above with adult worm antigen were seen in 75% and 60% of those with clinical filariasis due to B. *malayi* infection[2] and in 81.8% of patients with clinical lesions due to bancroftian filariasis.[13]

Enzyme-linked immunosorbent assays (ELISA) using soluble extracts of B. *malayi* microfilaria, infective larva or adult worms, have also been used. However, positive readings were obtained in 93–100% of subjects living in endemic areas, those with microfilareamia as well as those with clinical evidence of filariasis; 0–13% of those in non-endemic areas were also positive.[14] Thus the ELISA technique using crude extracts of the various stages of the parasite, is not as specific as the IFA assay. The test system will probably be more specific and useful if monoclonal antibodies against specific filaria epitopes are used, especially for the detection of circulating antigens.

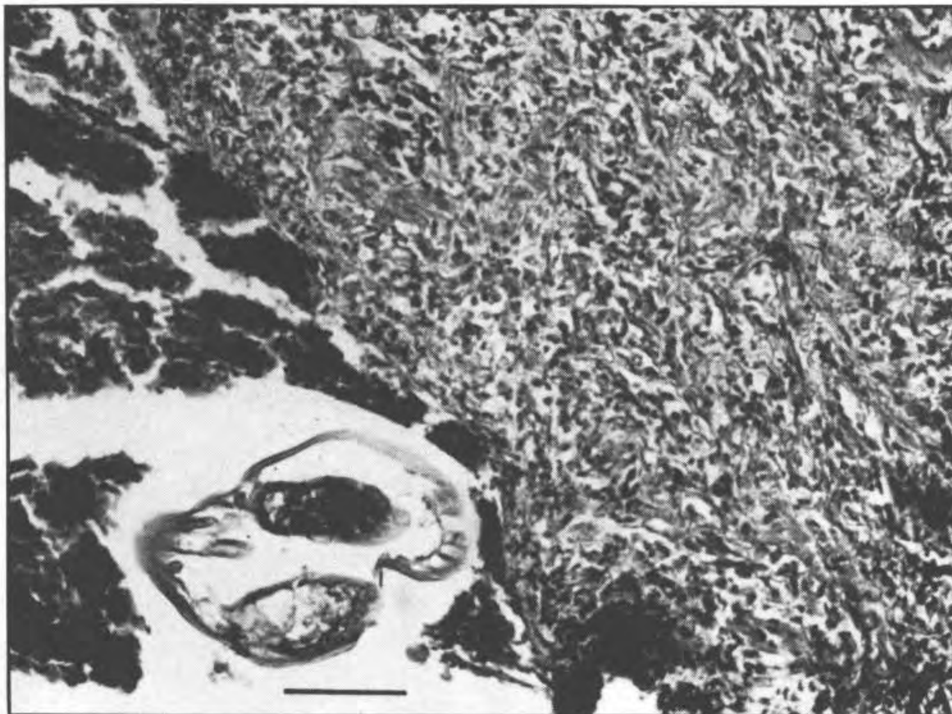

FIG. 4. Cross section of degenerating female ***Wuchereria bancrofti*** surrounded by inflammatory cells, in a nodule of the epididymis. Scale = 150 um.

DNA PROBES

Very specific DNA probes have now been developed against *B. malayi.*[15,16] However, except for their use as epidemiological tools in the identification of vector mosquitoes and possibly for mass surveys for microfilaraemics, they have a limited role for the diagnosis of clinical filariasis. As both *W. bancrofti* and *Brugia timori* are endemic in the region, specific probes for their identification must also be available. Furthermore, the existing probes are radioisotope labelled and thus have very short shelf lives, are expensive and very inconvenient to use.

ACKNOWLEDGEMENTS

I thank the Director, Institute for Medical Research, Kuala Lumpur, for permission to publish this paper.

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