Diagnosis of lymphatic filariasis: some issues and challenges

Mak JW *International Medical University, Sesama Centre, Plaza Komanwel, Bukit Jalil, 57000 Kuala Lumpur, Malaysia. (Correspondence: Dr Mak JW; e-mail: makjw@imu.edu.my)*

Abstract

Diagnosis of filariasis is based on clinical suspicion and confirmed by laboratory evidence of infection with the parasite. Detection of infection is needed in control programmes and for management of the individual patient. As the clinical spectrum of lymphatic filariasis is wide different laboratory diagnostic techniques may be required to detect different phases of the infection. Filariasis control programmes have rather specific requirements: we need to know the geographical distribution of endemic areas and tests are mainly needed to detect microfilaraemics and intensity of transmission. Control programmes based on selective or mass treatment of endemic populations require direct laboratory evidence of active infection. This may be achieved through demonstration of microfilaraemia or antigenaemia. Detection of infection in mosquitoes especially demonstration of the parasite infective stage in mosquitoes is needed to identify vectors and determine vectorial capacities. In the clinical setting, a greater range of techniques may be useful in assessing the stage of the infection or for differential diagnosis. For example, laboratory requirements for the diagnosis of tropical pulmonary eosinophilia and other forms of occult filariasis are different from that for detection of microfilaraemia in the patient presenting with acute adenolymphangitis.

Molecular approaches to diagnosis including the production of reagents for diagnostic assays have resulted in a variety of different assays for detection and evaluation of infection status. Many of these assays are extremely sensitive and specific but are not easily adapted to field use. Other assays, especially some commercial kits are convenient to use and can rapidly detect infection at the point of care. The challenge is to match the sensitivity and specificity of the assay to the appropriate situation and not to be overwhelmed by the perceived need to follow blindly practices useful in other health scenario. It is important to determine the appropriate assays for the different purposes needed by healthcare workers in control programmes and in clinical practice. Perhaps it is opportune for us to produce practice guidelines on what tests are appropriate in the different health situations. Outcomes research based on cost effectiveness, client satisfaction, efficiency and accuracy, etc. utilising meta-analyses of data from health and medical practices as well as clinical trials can assist and should be carried out.

Key words: clinical diagnosis, molecular assays, rapid diagnostic tests, outcomes research

Introduction

In the clinical situation, the presenting features and history of living in an endemic area can usually provide a provisional diagnosis of filariasis. This will need sufficient knowledge on the geographical distribution of lymphatic filariasis. In most situations the classical demonstration of microfilaria in the blood or other body fluids is attempted using direct detection or after microfilarial concentration using Knott's concentration or filtration of the diluted sample through a 3 μm pore size polycarbonate filter. These techniques have been the mainstay for the laboratory diagnosis in most diagnostic laboratories as well as for control programmes. More specialised techniques have been utilised to overcome some of the perceived limitations of these direct demonstration of microfilaria for diagnosis. Tests based on direct demonstration of the parasite suffer from poor sensitivity when the sample is collected during an inappropriate time for parasite strains that show marked periodicity. However for strains that do not have a marked periodicity and for aperiodic strains this would not be too much of a problem. There are clinical situations too when microfilaria may not be detected in the peripheral blood stream. These include pre-patent infections, single sex parasitic infections, obstruction of lymph flow from the sites of adult worms to the circulation, and when microfilariae are not being produced due to drug action and/or burnt out infections. Indirect techniques were then developed as correlates of infection. These included detection of specific anti-filarial sub-class antibodies,

antigen-antibody complexes, and circulating soluble antigens. These techniques had the advantage of ease of sample collection but were not very sensitive and specific. These have not been routinely used in control programmes, as they require greater technical skills to carry out and availability of appropriate reagents. Furthermore none of the older tests could be used to discriminate the stage of the infection accurately. These problems especially those related to production of specific reagents for the diagnosis of bancroftian filariasis resulted in intense research to develop more sensitive, specific and convenient techniques for the diagnosis of lymphatic filariasis. This has resulted in a large variety of newer techniques, among which was detection of parasite specific nucleic acid sequences. These newer techniques have increased the sensitivity and specificity of parasite detection but an important issue will be appropriateness of each technique for defined health delivery situations. It is also necessary to make recommendations based on evidence and outcome measures on what should be recommended for each health care activity. This review will therefore attempt to trace the development of the different groups of assays and evidence for their appropriate use in various clinical and epidemiological situations; examine the results of studies in support of the claims made and make recommendations on what would be the appropriate tests to use for the various health care scenarios.

Antibody Assays

In general an antigen detection assay would be preferable to an antibody detection assay. Antigen detection assays for *B. malayi* infection are not as well developed as those for bancroftian filariasis. Specific antibody assays have been utilised for some time mainly to differentiate occult filariasis from other medical conditions. Antibody based assays may also be useful for epidemiological assessment. A disadvantage in these assays is mainly due to the use of undefined antigens in most of these assays.

Dimock *et al.* (1996) have shown that in bancroftian filariasis significantly elevated antifilarial immunoglobulin G4 (IgG4) levels were associated with antigenaemia, whereas microfilaraemia was associated with significantly decreased antifilarial IgG2 levels.

Zhang *et al.* (1999) found brugian filariasis patients had significantly higher IgE and lower IgG4 levels to adult worm antigens than people with asymptomatic microfilaraemia. IgE antibodies were found to bind a subset of soluble L3 antigens bound by IgG, preferentially those of 200, 97, 68 and 58 kDa.

Shared Filarial Antigens as Diagnostic Reagents

It is well known that filarial parasites share common antigens and this has been taken advantage of by researchers in obtaining sufficient antigens for study. The lack of antigenic material for use in diagnosis is particularly acute for bancroftian filariasis, which accounts for 90% of all lymphatic filarial infections in the world. Animal filarial parasites are frequently used as sources of diagnostic reagents as these have cross-reacting antigenic epitopes. For example, an antigen fraction isolated from the aqueous-insoluble components of adult *Setaria digitata* (from cattle) yielded a 1.56×10^5 molecular mass fraction (named $Dssd_1$) which appeared to be similar to antigens expressed on the surface of *W. bancrofti* microfilaria (Bal & Das, 1999). Specific IgM antibody titres to this surface antigen were found to be highest in endemic normals followed by those with chronic pathology and asymptomatic microfilaraemics. Interestingly, IgG subclass antibodies were different in composition in these three groups. The cytophilic IgG1 and IgG3 specific antibodies to this surface antigen were highest in those with chronic pathology and in endemic normals respectively. In contrast the non-cytophilic specific subclass antibody IgG4 was mainly seen in the asymptomatic microfilaraemics. The specific IgG2 subclass antibody was also seen in all the three groups. On DEC treatment there was an increase and change in composition of these subclass antibodies. There was an increased IgG3 and IgG1 response associated with a decline in IgG4 following successful treatment (Bal & Das, 1999). It is therefore postulated that the cytophilic subclass IgG1 and IgG3 antibodies to Dssd1 are associated with clearing of microfilariae form the circulation (Bal & Das, 1999).

Cuticular antigens of molecular weights 130 kDa and 52 kDa derived also from *W. bancrofti* have been shown to be present in the serum of asymptomatic microfilaraemic *W. bancrofti* subjects. These antigens in microfilaraemics are postulated to be derived from *W. bancrofti* adults. They can detect and differentiate microfilaraemic subjects from endemic normals in a dot-blot assay based on the lesser antigen concentration for detection of the former (Wickremanayake *et al.*, 2001).

A 66-kDa soluble extract of *B. malayi* microfilaria was used in dot blot assay to detect antibodies in bancroftian filariasis (Balaji Ganesh *et al.*, 2001). This simple and cheap assay was found to be 90% sensitive when compared against the blood film positives and had a presumed specificity of 80% as it detected as positives 5 out of 25 endemic normals.

A 43-kDa molecule circulating filarial antigen fraction (CFA_2-6) present in the plasma of microfilaraemic subjects of bancroftian filariasis patients have been shown to be present in the somatic extracts of adults, infective larvae (L3) and microfilariae (Mf) and the surface of the later two stages of *Brugia malayi* (Vasu *et al.*, 2000). This antigen in microfilaraemic bancroftian patients is highly

reactive specifically to endemic normal groups (Cheirmaraj *et al.*, 1991) and has been shown to induce protective immunity to challenge with *B. malayi* L3 in immunized jirds. This antigen appears to be associated with protective immunity and may be useful in identifying those exposed individuals in endemic localities who are 'immune'.

The 43-kDa molecule present in the plasma of microfilaraemic bancroftian filariasis is also present in somatic extracts of mf, L3 and adults as well as the surface of LI and L2 of B. malayi. As this molecule is highly reactive specifically to endemic normals this may be useful to identify 'immunes' (Cheirmaraj *et al.*, 1991).

Recombinant Proteins as Diagnostic Reagents

In view of the difficulty in obtaining *W. bancrofti* antigens as diagnostic reagents, recombinant proteins have been generated for the purpose. Rao *et al.* (2000) identified from a *W. bancrofti* L3 cDNA library the gene *Wb-sxp*-1 encoding a basic protein with a calculated molecular mass of 20.8 kDa. *Wb*-SXP-1 was 85% identical to the SXPI protein described from *B. malayi* (Bm-SXP-1). The *Wb*-SXP-1 sequence also showed significant identity with proteins described from *B. pahangi. Onchocerca volvulus, Acanthochilonema vitea. Ascaris suum. Loa loa, Litomosoides sigmodontis* and *Caenorhabditis elegans.* The presence of a number of invariant and conserved residues in all of these nematode-derived molecules suggests that *Wb*-SXP-1 is a member of a new protein family. A recombinant form of *Wb*-SXP-1 was produced and it was determined that the anti-*Wb*-SXP-1 antibody response in patients with *W. bancrofti* infections was restricted to the IgG4 subclass. An anti-*Wb*-SXP-1 IgG4 ELISA was developed and this assay was found to be 100% sensitive for patients with patent *W. bancrofti* infection. Sera from individuals experiencing chronic pathology, endemic normals or patients with non-filarial nematode infections had no detectable IgG4 against *Wb*-SXP-1. While patients with patent *Onchocerca volvulus* infections were uniformly negative in the *Wb*-SXP-1 assay, 40% of sera from patent *Loa loa* infections were positive. When *Bm*SXP-1 was used as the antigen under identical conditions, the assay was 88% specific for patent *W. bancrofti* infections and the antigen was recognized by antibodies from both *O. volvulus* and *L. loa* infections. The results strongly suggest that, for certain diagnostic filarial antigens, the use of same-species molecules can enhance the specificity of diagnostic tests.

Recently a recombinant *B. malayi* antigen (*BmR1*) based ELISA assay (*Brugia*-ELISA) has been developed and shown to be 95.6 – 100% specific and 96 –100% sensitive in the detection of active infections (Rahmah *et al.*, 2001). Although it crossreacted with *Brugia timori* (2 microfilaraemics tested) and *W. bancrofti* (54.5% of 22 microfilaraemics tested) infections, it could detect all endemic normals that were positive on PCR-ELISA.

A recombinant chitinase antigen was also found to be superior to microfilarial extract in detecting antibodies *W. bancrofti* microfilaraemic subjects and *B. malayi* microfilaraemic jirds. The antigen was reactive with 100% specimens compared to only 87% with microfilarial extracts (Wang *et al.*, 1999).

Yazdanbakhsh *et al.* (1995) used two recombinant *Brugia* spp. proteins to study isotype antibody responses in brugian filariasis. The C-terminal portion of the heat shock protein 70 (Bpa-26) representative of a cytoplasmic protein and a single unit of the tandem repeats of a *Brugia* polypeptide (BpL-4), a secretary glycoprotein were used. Microfilaraemic individuals show high-level antifilarial IgG4 responses but generally little specific IgE antibody to total parasite extract. In most patients with elephantiasis the IgE levels are high but IgG4 levels low. The antibody isotype response to defined antigens showed some interesting results. In elephantiasis there was an increased IgG3 response to Bpa-26. IgG3 response to BpL-4 in elephantiasis and microfilaraemic were similar, both being greater than those in asymptomatic amicrofilaraemics, suggesting that the existence of large parasite loads drives IgG3 responses to BpL-4. As IgG3 antibodies to Bpa-26 were significantly elevated only in elephantiasis patients compared to asymptomatic microfilaraemics and endemic normals, they postulated that this response is associated with pathology.

Appropriate Antigens for Antibody Detection Assays

From the above we can conclude that animal filarial parasites may be useful sources of diagnostic reagents in tests for antibody detection of bancroftian filariasis. *S. digitata* which is a common filarial parasite of cattle has been shown to have antigenic epitopes similar to those of *W. bancrofti.* The aqueous insoluble fraction ($Dssd_1$) of the parasite which is antigenically similar to the surface antigens of *W. bancrofti* microfilaria (Bal & Das, 1999), can be used in an IgM-ELISA or an IgG2-ELISA assay to detect specific antibodies in endemic normals, microfilaraemics and those with chronic pathology. The IgG4-ELISA, which is seen mainly in microfilaraemics, can be used together with the IgG1-ELISA and IgG3-ELISA to monitor the effectiveness of therapy of microfilaraemis. There should be significant drop of IgG4-ELISA titres and corresponding increases in IgG1-ELISA and IgG3-ELISA titres on microfilarial clearance. Two other *S. digitata* derived antigens have been shown to have potential as diagnostic reagents; a 130 kDa and 52 kDa cuticular antigens are also present in the serum of bancroftian microfilaraemics and are believed to be from adult *W. bancrofti* worms. Interestingly, a dot-blot assay has been developed which can differentiate microfilaraemic subjects from endemic normals (Wickremanayake *et al.*, 2001). Thus we have available from *S. digitata* antigenic correlates of the *W. bancrofti*

microfilarial surface antigen ($Dssd_1$) and adult worms (130 kDa and 52 kDa cuticular antigens). All three can be used in tests for detection of bancroftian microfilaraemics. The IgG1-ELISA and IgG3-ELISA with the $Dssd_1$ antigen will be useful for detection of infected amicrofilaraemics and those with chronic pathology. Thus these assays should be explored further for laboratory diagnosis and for epidemiological use. However, it should be noted that a 20.8 kDa recombinant protein (*Wb*-SXP-1) expressed by a fairly well conserved filarial gene (*Wb-sxp-1*) specifically detected all patent *W. bancrofti* infections in an IgG4-ELISA (Rao *et al.*, 2000). The test was negative for endemic normals, chronic pathology, and other nematode infections. This apparent specificity through the use of homologous antigen must be confirmed. Monoclonal antibodies against these antigens could also be developed for use in antigen detection assays.

Antigen Detection Assays

Antigen detection assays are attractive as a positive test result indicates a current or recently 'cured' (successfully treated or parasite destruction and elimination due to host responses or combination of host response and treatment) infection. It may not be possible to detect recently acquired infections still at the pre-patent stage before antigen levels are detectable in circulation.

Commercially available antigen detection kits are available for detection of *W. bancrofti* infections. They are available in ELISA (Tropical Biotechnology Pty Ltd (TropBio) Australia, or rapid immunochromatographic card test format (ICT Diagnostic, Balgowlah, NSW, Australia). The ELISA kits utilize a monoclonal antibody against *Onchocerca gibsoni* (Og4C3) to detect bancroftian circulating filarial antigen in a sandwich ELISA format. The ICT card test also utilizes a monoclonal antibody AD.12 that has a high sensitivity and specificity to *W. bancrofti* antigen.

The TropBio ELISA kits for serum and filter paper and the ICT kit were all shown to be 100% sensitive in detecting bancroftian microfilaraemic subjects (Simonsen & Dunyo, 1999). The filter paper version of the ELISA also showed a statistical positive correlation with microfilarial density. All three tests could detect circulating antigens in endemic normals, positives being seen in 10, 9 and 8 out of 27 examined with the serum TropBio ELISA, filter paper TropBio ELISA and ICT tests respectively. As the assay probably detected circulating antigens from adult worms these results show that many endemic normals could in fact harbour adult worms but undetectable microfilaraemia.

The ICT card test, which is applicable at the point of care, has been evaluated in various bancroftian endemic areas (Freedman *et al.*, 1997; Bhumiratana *et al.*, 1999). It was found to be 100% specific and able to discriminate *W. bancrofti* from *B. malayi* microfilaraemic subjects as well as those with common intestinal helminths and *Opisthorchis viverrini* infections (Bhumiratana *et al.*, 1999). It could detect antigenaemia in 13.7% of endemic normal subjects. Overall it detected 20.0% positive compared to 5.8% positive with the thick blood smear and clinical and recall techniques and 5.3% with the capillary tube detection of microfilaraemia. The ICT kit was shown to be 100% sensitive in detecting bancroftian microfilaraemic subjects (Simonsen & Dunyo, 1999).

The ICT was also found to detect more positives for antigenaemia in bancroftian filariasis endemic areas when compared to counting chamber and thick blood smear technique for microfilaraemia (Onapa *et al.*, 2001). In Alebtong, Lwala and Obalanga communities the ICT was positive in 29%, 18% and 30% respectively while the counting chamber technique gave positives of 18%, 9% and 21% respectively. The blood film technique gave positive rates of 26%, 10% and 22% respectively for *W. bancrofti* microfilariae.

Antigen detection assays are also increasingly being utilised in studies to evaluate the effectiveness of chemotherapy in clinical trials (Ismail *et al.*, 2001).

Oligonucleotide Probes, PCR and Related Techniques

In the last 15 years or so there has been rapid development and use of oligonucleotide probes and polymerase chain techniques for detection of filarial parasites (Sim *et al.*, 1986; Gunawardene *et al.*, 1999). Radioactive probes have been replaced by more convenient non-radioactive probes utilising chemiluminescent-based detection, and these were shown to be extremely sensitive, able to detect a single microfilaria and infective larva (L3). However, they are more complex and expensive to use and in the case of the *W. bancrofti* probe (WBB1-5), though based on repetitive DNA sequences presumed to be specific for the parasite, has been shown to cross reacts with *B. malayi* (Gunawardene *et al.*, 1999).

A rapid PCR-ELISA that included an internal standard for the detection of *Brugia Hha I* repeat was only 86% positive when filter-paper samples of periodic *B. malayi* were used when compared to results with filtration of 1 ml of blood (Kluber *et al.*, 2001).

A nested PCR on finger-prick blood samples collected on filter papers was shown to be very sensitive using primers based on the highly repetitive *B. malayi Hhal* sequence. It could detect all 30 microfilaraemic positives out of 145 samples examined, and a further 13 negative on microscopy (Cox-Singh *et al.*, 1999). The simple blood collection and one-tube DNA extraction method from samples as described made this method attractive for specialised studies.

Currently used protocols for the extraction of filarial parasite DNA from mosquito samples are tedious and involve extensive use of expensive and hazardous chemicals. Vasuki *et al.* (2001) developed a simple and efficient method for the extraction of DNA from mosquitoes infected with filarial parasite, *Brugia malayi*. It was found to be as efficient as the current procedure for the extraction of DNA from a single microfilaria in pools of 25 mosquitoes and the DNA was suitable for polymerase chain reaction (PCR) amplification. The method involved drying and crushing the mosquitoes to a powder, which was then homogenized in TE buffer, and the supernatant used for the PCR assay. Dot-blot hybridization confirmed the specificity of the PCR amplified fragment. The DNA extracted by this method was stable for about 1 year. When compared with the standard method, the cost of a single PCR reaction, inclusive of DNA extraction, was reduced by 50% and the hands-on time was minimized fivefold. Hence, this simple TE-based method is rapid, safe and also cost-effective in assessing the *B. malayi* infection in pools of vector mosquitoes. However, the procedure for DNA extraction as described will not be useful to detect infective mosquitoes nor incriminate vectors. It is well known that many mosquitoes that take in microfilariae may not be able to support their further development to the infective stage and thus would not be a suitable vector. Positives would therefore only mean the presence of microfilariae and/or developing (L1, L2) and infective larvae.

Clinical Diagnosis

It will not be complete if we do not review some recent advances in diagnosis based on detection of adult worms. Dreyer and co-workers have been credited with using and developing ultrasound for the detection and location of the adult lymphatic filarial parasite. They have increased the detection rate of live adult *W. bancrafti* worms in the scrotal area of microfilaraemic patients from 80% to 88% by using provocative treatment with DEC 12 hrs to 7 days prior to ultrasound examination (Dreyer *et al.*, 1999). They further suggested that in the ultrasound should be included in the panel of tests to characterize 'endemic normals'.

Geographical Information System for Lymphatic Filariasis Mapping

Health managers need accurate information on the distribution, burden and the determinants of transmission of the infection in the country to strategise and prioritise allocation of resources for filariasis control. The view that lymphatic filariasis can potentially be eradicated (CDC, 1993) is a powerful incentive to explore strategies which will allow national programmes to decide whether their available resources would be sufficient to achieve this. An exciting and potentially useful tool for mapping endemic areas of lymphatic filariasis for use in control programmes has been recently proposed. A recent WHO report on a workshop outlined the usefulness and proposed methods to be employed for the rapid geographical assessment of bancroftian filariasis (WHO, 1998).

Lindsay & Thomas (2000) used the principle that climate is a first-order determinant of vector-borne diseases distribution and applied this to bancroftian filariasis in Africa and Egypt. Logistic regression analysis of climate variables predicted with 76% accuracy whether sites had microfilaraemic patients or not (based on previous studies). They used this logistic regression equation in a geographical information system to map the risk of bancroftian filariasis across Africa and Egypt and correctly predicted 88% and 84% infected sites respectively. They further postulated that by overlaying risk maps so determined over population maps, estimates of people exposed can be made thus providing the sampling frame for designing and conducting filariasis surveys for control purposes. Further studies along these lines to map endemic areas according to risks will provide control programme managers with data to prioritise and allocate scarce resources for control of the infection. Similar studies should be carried out for brugian filariasis to determine whether the assumptions made for bancroftian filariasis are also applicable.

Conclusion

The large numbers of existing tools available for detection of lymphatic filariasis should be evaluated rationally in order that appropriate techniques are applied to specific situations. It is important to differentiate those that are most useful for control programmes and those that are more appropriate for clinical diagnosis and management of patients. Sometimes a combination of tests will be appropriate and should be used. In other situations the technique recommended may not be the most sensitive available but because of logistic and economic factors can be considered as appropriate. Thus the geographical information system to assess lymphatic filariasis distribution can be a powerful tool in assisting control programme managers prioritise and allocate national resources for control of the infection.

Of particular concern are the opportunities that are lost when results of studies are not utilised for development of better diagnostic tools for various clinical or epidemiological situations in filariasis. These are more so when studies from endemic countries have not been given due emphasis and follow-up because of economic and other factors. There should be a conscious effort to 'mine' research results and identify potential leads for support

and follow-up.

There is also a need for consensus statements and guidelines based on meta-analyses of well-designed studies to assist health workers in the choice of the appropriate or combination of tests for different health situations.

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