

Proposed panel of diagnostic criteria, including the use of ultrasound, to refine the concept of ‘endemic normals’ in lymphatic filariasis

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Summary

Although living adult *Wuchereria bancrofti* worms can be detected by ultrasound examination of the scrotal area in approximately 80% of men infected with this filarial parasite, the location of the adult worms in the remaining 20% remains unclear. To determine this, 32 individuals who had *W. bancrofti* microfilaraemia but no adult worms detectable on ultrasound were treated with diethylcarbamazine (DEC), either with a single 6 mg/kg dose ($n = 13$) or with a 12-day course of 6 mg/kg per day ($n = 19$). They were then monitored with serial physical and ultrasound examinations. Thirteen (41%) subjects developed small, single scrotal nodules 12 h to 7 days after treatment; this rate was unaffected by the dose of DEC. No nodules were detected outside the scrotal area. All 5 men with lymphangiectasia suspected on ultrasound before treatment developed scrotal nodules, compared to 8 (29.6%) of 27 men without ultrasonographic evidence ($P = 0.006$). Thus, using both ultrasound and ‘provocative’ treatment with DEC, adult *W. bancrofti* can be detected in the scrotal area of an estimated 88% of infected men. Because no single diagnostic test for *W. bancrofti* infection is completely sensitive, a panel of tests, including ultrasound, is proposed to identify with greater accuracy ‘endemic normals’ for immunological and epidemiological studies.

keywords diethylcarbamazine, adult worm, filariasis, *Wuchereria bancrofti*, ultrasound

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Introduction

In men, scrotal hydrocele is the most common chronic clinical manifestation of bancroftian filariasis (Wijers 1977; Gyapong *et al.* 1994). The pathogenesis of hydrocele associated with lymphatic filariasis remains obscure (Ottesen 1992), but the high frequency of urogenital disease in men with bancroftian filariasis is almost certainly related to the apparent preference of adult *Wuchereria bancrofti* for the scrotal area. Clinical, histological and ultrasonographic evidence supports this notion. In men, the scrotal area is by far the most frequent site of nodules following treatment with diethylcarbamazine (Chen 1964); biopsies of these nodules reveal dead adult *W. bancrofti* surrounded by inflammatory infiltrates (Dreyer *et al.* 1995; Figueredo-Silva *et al.* 1996). Further, at least 80% of men with living adult worms detectable in the scrotal area by

ultrasound also have *W. bancrofti* microfilariae in peripheral blood (Noroes *et al.* 1996a). The adult worms exhibit a characteristic pattern of movement known as the filaria dance sign (Amaral *et al.* 1994) and the location of these adult worm ‘nests’ remains remarkably stable (Dreyer *et al.* 1994b).

In the approximately 20% of men with *W. bancrofti* infection who have no detectable filaria dance sign on ultrasound examination, the location of the adult worms remains unclear. It is possible that adult worms are present in the scrotal area, but remain below the detectable limit. Previous work has shown that with a 7.5-MHz probe, adult worms cannot be reliably detected if the diameter of the lymphatic vessels is less than 1 mm (Noroes *et al.* 1996b). Alternatively, the adult worms may live outside the scrotal area. To help resolve this issue, we provided DEC treatment to men who were known to be infected with *W. bancrofti* (they were

microfilaraemic) and who had repeatedly tested negative in ultrasound examinations of the scrotal area for evidence of the adult worm.

Materials and methods

Informed consent was obtained from all subjects before enrolment in the study, and the study protocol was approved by the Ethics Committee of Hospital das Clínicas, Universidade Federal de Pernambuco, Recife, Brazil. Thirty-two adult men, all residents of Greater Recife, were recruited. All had visited the filariasis clinic at Centro de Pesquisas Aggeu Magalhães/FIOCRUZ, in Recife for screening or treatment of *Wuchereria bancrofti* infection. Men were enrolled in the study if *W. bancrofti* microfilariae were detected in their peripheral blood; if no movements characteristic of the adult worm, the filaria dance sign, were detected on ultrasound examination of the scrotal area, if they denied having taken antifilarial drugs (diethylcarbamazine or ivermectin) and having had any episodes of acute adenolymphangitis, and if no scrotal nodules or hydroceles were detected on urogenital examination.

To estimate microfilarial density, 1 ml of venous blood was collected between 2300h and 0100 h and filtered through a 3 µm polycarbonate filter (Nuclepore Corporation, Pleasanton, CA). The filter was then placed on a slide, stained with Carrazzi-haematoxylin, and examined microscopically. Pretreatment ultrasound examinations of the scrotal area were conducted independently by two ultrasonographers using a 7.5-MHz transducer as previously described (Amaral *et al.* 1994). Each ultrasonographer examined the scrotal area on three occasions during a single week in an effort to detect the filaria dance sign and assess the presence of lymphangiectasia.

After informed consent was obtained, subjects were treated with a single 6 mg/kg dose of DEC ($n = 13$) or a standard 12-day course of 6 mg/kg per day ($n = 19$) as recommended by the World Health Organization (WHO 1992). Patients were interviewed regarding local adverse reactions and physical and ultrasound examinations were performed 24, 48, and 72 h after the first day of treatment and then once a week for 4 weeks. Three men who developed scrotal nodules after treatment underwent biopsy of these nodules under local anaesthesia for standard histological evaluation. Differences in proportions were evaluated for statistical significance with the two-tailed Fisher exact test, and the Wilcoxon Rank Sum test was used to compare differences in distributions of continuous variables.

Results

The 32 men ranged in age from 19 to 56 years (mean 24 years). Geometric mean microfilarial density was 99 per ml

of blood, with a range of 1–2220. In 5 men lymphangiectasia was suspected on ultrasound examination, in 3 on the left side and in 2 on the right. Thirteen subjects (40.6%) developed small, single scrotal nodules, which were first detected 12 h to 7 days after onset of treatment (mean, 4.5 days). Six nodules were detected on the left side and seven on the right. No nodules were detected in other parts of the body, and none of the men complained of pain or tenderness outside the scrotal area. The treatment regimen did not lead to significant differences in the proportion who developed scrotal nodules (38.5% of single-dose subjects and 42.1% of multiple-dose patients), or in the mean interval between onset of treatment and detection of scrotal nodules (4.4 days and 4.6 days, respectively). Suspected pretreatment lymphangiectasia on ultrasound examination was significantly associated with the development of scrotal nodules after treatment. All five men with suspected lymphangiectasia on ultrasound developed scrotal nodules, compared to 8 (29.6%) of 27 men with no ultrasonographic evidence of lymphangiectasia in the scrotal area ($P = 0.006$).

Three men who developed scrotal nodules underwent biopsy. The two nodules that were biopsied 14 and 35 days after onset of treatment showed degenerating adult worms. The third biopsy, collected 132 days after onset of treatment, showed calcified adult worms. None of the 32 men had severe local or systemic adverse reactions, and none of the 3 men who underwent biopsies developed complications or problems associated with the procedure. All subjects were in good health 18 months after treatment.

Discussion

In addition to being the drug of choice for a variety of filarial infections for more than half a century (Hawking 1979; Mackenzie & Kron 1985), DEC has also proved useful as a diagnostic tool. In patients suspected of having onchocerciasis, a single low dose of DEC produces characteristic systemic signs and symptoms known as the Mazzotti reaction (Mazzotti 1948). The occurrence of these reactions following treatment with DEC has since been used as a diagnostic aid for *Onchocerca volvulus* infection. In persons with *W. bancrofti* infection, administration of a single dose of DEC during the day dramatically increases concentrations of microfilariae in the peripheral blood within 30 min. Known as the 'provocative day test', such treatment with DEC allows for diagnosis of *W. bancrofti* microfilaraemia during daylight hours, even in areas where microfilaraemia is nocturnally periodic (Wong & Chong 1967; Sullivan & Hembree 1970; McMahon *et al.* 1979; Sabry 1988). The adulticidal effect of DEC on *W. bancrofti* has also been useful diagnostically. After treatment with DEC, men who are infected with this parasite frequently develop inflammatory nodules in the

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scrotum at the site of the dead adult worm (Dreyer *et al.* 1994a). These nodules may be painful and tender, or they may cause no symptoms (Dreyer *et al.* 1995; J. Noroes, personal communication). Treatment with DEC followed by examination for nodules has been used in epidemiological and immunological studies to rule out adult worm infection in reputed 'endemic normals', i.e. individuals who remain asymptomatic and amicrofilaraemic despite apparent exposure to the parasite (Freedman *et al.* 1989; Day 1991).

Despite the availability of several diagnostic techniques including filtration of larger volumes of peripheral blood, circulating filarial antigen assays, detection of adult worm movement on ultrasound and the DEC provocative test, it is becoming increasingly clear that no single test can identify 'endemic normals' (i.e. 'putative immune' individuals) with certainty. When more than one such test is used, the number of persons in filariasis-endemic areas who can truly be classified as endemic normals is smaller than previously realized (Day 1991; Lammie *et al.* 1994). Persons initially thought to be endemic normals have been found, on more thorough investigation, to have ultralow levels of microfilaraemia or to be amicrofilaraemic adult worm carriers (Dreyer *et al.* 1996; Rocha *et al.* 1996). This study highlights the frequent occurrence of adult worms in the scrotal area and certain limitations of ultrasonography in detecting these worms. Although ultrasound may be the only tool that detects infection in some men (i.e. those who are amicrofilaraemic and have no detectable circulating filarial antigen) (Rocha *et al.* 1996; Noroes *et al.* 1997), up to 20% of microfilaraemic men have no evidence of adult worm infection on ultrasound examination of the scrotal area (Noroes *et al.* 1996a). In our current study, 41% of such men harboured living adult *W. bancrofti* whose presence was revealed by treatment with DEC.

The actual percentage of infected men with adult worms in their scrotal lymphatics is likely to be considerably higher. Earlier studies have shown that only 45–50% of men who are infected with *W. bancrofti* develop scrotal nodules after treatment with DEC (Dreyer *et al.* 1995; Noroes *et al.* 1997), even when the adult worms are detected by ultrasound (Noroes *et al.* 1997). Thus, the susceptibility of adult *W. bancrofti* to DEC is considerably less than 100%, and perhaps half of adult worm nests would not be expected to be detected by provocative treatment with DEC.

Three of the 19 men who did not develop nodules in response to DEC returned to the clinic on their own initiative after the 4-week follow-up period. We used this opportunity to perform physical and ultrasound examinations. One man developed a painful scrotal mass 6 months after treatment (indicative of spontaneous adult worm death, probably unrelated to the treatment), and a scrotal nodule was detected on physical examination. In two men, the living adult

worms first became detectable on ultrasound examination of the scrotal area 8 and 12 months after treatment. With a 7.5-MHz transducer, the practical limit of detection of the filaria dance sign is reached when the lymphatic vessel diameter approaches 1 mm (Noroes *et al.* 1996b), close to the normal diameter of lymphatic vessels in the spermatic cord (Moller 1980). Lymphatic dilatation appears to be a universal finding in men with a detectable filaria dance sign (Noroes *et al.* 1996b), and unpublished observations suggest that the dilatation increases as long as living adult worms are present (G. Dreyer, unpublished data). Thus, it seems likely that all three of these men had adult *W. bancrofti* in the scrotal area at the time of the study, but that the vessel diameter had not yet progressed to the point where the adult worm nest could be visualized by ultrasound. We cannot rule out the possibility, which we consider much less likely, that these men were reinfected after the 4-week period of the study, and that the findings at 6, 8, and 12 months after treatment were the result of that.

The DEC provocative test results in this study support the notion that the scrotal area is the most common site for living adult *W. bancrofti*, even in infected men with negative ultrasound results. None of these men developed nodules outside the scrotal area. Even in men in whom adult worms are detectable by ultrasound, treatment with DEC may provoke nodules in the scrotal area, but in different sites where no filaria dance sign may have been detected. This was observed in five men aged 19–29 years, who were initially screened for this study and found to have unilateral filaria dance signs. Treatment with DEC did not change the ultrasonographic image, but all five men developed scrotal nodules within 6 h to 7 days on the side contralateral to where the filaria dance sign was visualized.

Although the scrotal area appears to be the preferred site of adult *W. bancrofti* in men, it is also clear that, on rare occasions, living adult worms reside in other locations including in the lymphatic vessels in the crural and inguinal area and in the axillary and epitrochlear lymph nodes (Dreyer & Piessens 1999; Dreyer *et al.* 1999). The apparent preference of *W. bancrofti* for the scrotal area remains unexplained. To date, ultrasonographic studies of the other major human lymphatic filarial worm, *Brugia malayi*, have not been reported, so the preferred site of adult *B. malayi* is not known. Such studies would be of interest because, in contrast to *W. bancrofti*, *B. malayi* infection is not known to cause chyluria or scrotal pathology.

This study reinforces the importance of suspected lymphangiectasia on ultrasonographic examination of the scrotum as a specific marker for current or past *W. bancrofti* infection. All 5 men with suspected lymphangiectasia developed scrotal nodules, which confirmed the presence of adult worms in that location, compared to only 8 (30%) of

27 subjects without lymphangiectasia. This observation is consistent with an earlier study in which all 78 men who had *W. bancrofti* detectable by ultrasound also had lymphangiectasia (Noroës *et al.* 1996b). The specificity of the relationship between lymphangiectasia in the intrascrotal area and current or past *W. bancrofti* infection argues for the absence of lymphangiectasia as a new criterion for identifying potential endemic normals.

This study also confirms earlier findings that the adulticidal efficacy of a single 6 mg/kg dose of DEC is similar, if not equivalent, to that of a 12-day course of the drug (Noroës *et al.* 1997). At any given time, adult *W. bancrofti* appear to be either susceptible or not susceptible to DEC; worms that are not susceptible to a single 6 mg/kg dose of DEC do not appear to respond to higher doses. Populations of both susceptible and non-susceptible worms can be found in the same person (Noroës *et al.* 1997).

Our data have implications for immunological and epidemiological studies of endemic normals. Although this study focused on the usefulness of the DEC provocative test and on the limitations of ultrasound to detect adult worms, 19 men were described who, at the time of the study, had no adult *W. bancrofti* worms detected by either of these techniques, even though they were microfilaria-positive. Several sensitive diagnostic tests are now available, including filtration of venous blood collected at night, assays to detect circulating filarial antigen, detection of adult worms by ultrasound, and the DEC provocative test, but none of these tests is 100% sensitive for adult worm infection (Dreyer *et al.* 1994a, 1996; Lammie *et al.* 1994; Rocha *et al.* 1996). As the field use of more sensitive tests has proliferated, the original, relatively clear notion of endemic normals as 'individuals living in endemic regions (i.e. exposed to infected mosquitoes) but with absolutely no clinical or parasitological evidence of infection' (Ottesen 1984) has been blurred. For example, in three recent studies, all microfilaria-negative subjects without obvious clinical disease were considered to be endemic normals, regardless of whether circulating filarial antigen was detected in their blood (Weil *et al.* 1996; Faris *et al.* 1998; Lalitha *et al.* 1998). For lymphatic filariasis, we believe that the term 'endemic normal' should be reserved for persons who, on the basis of a panel of several tests, appear to be uninfected despite long-term residence in an area of intense exposure to the parasite, and who have no evidence of either clinical lymphatic disease or subclinical lymphangiectasia. Because they already have evidence of subclinical disease, men who have suspected or readily detected lymphangiectasia on ultrasound examination of the scrotal area should not be considered endemic normals, even if they have no other evidence of current *W. bancrofti* infection. We suggest that for men in filariasis-endemic areas to be considered as potential endemic normals, they should have no

microfilaria detected in filtration of at least 16 ml of blood; negative tests for circulating filarial antigen; no filaria dance sign detected by ultrasound; no lymphatic nodules following a provocative dose of DEC; and no ultrasonographic evidence of lymphangiectasia in the intrascrotal area.

Finally, the findings of this study and the lack of 100% sensitivity of diagnostic tests lend support to the recommendation of the National Health Foundation of the Brazilian Ministry of Health that persons living in filariasis-endemic areas be tested every six months for microfilaraemia. For healthy adult males at risk of *W. bancrofti* infection, half-yearly ultrasound examinations of the scrotal area may be useful to identify and treat early infections before microfilaria appear in the blood and lymphatic damage and progression of subclinical disease occurs.

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