ULTRASONOGRAPHIC DETECTION OF LIVING ADULT WUCHERERIA BANCROFTI USING A 3.5-MHZ TRANSDUCER

GERUSA DREYER, ABIEL SANTOS, JOAQUIM NOROES, FERNANDO AMARAL, AND DAVID ADDISS

Departamento de Parassitologia, Centro de Pesquisas Aggeu Magalhaes-FIOCRUZ, Recife, Brazil; Hospital das Clinicas, Universidade Federal de Pernambuco, Recife, Brazil; Mediax, Memorial Imagem e Diagnostico, Recife, Brazil; Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract. Adult Wuchereria bancrofti can be readily detected by ultrasound in the lymphatic vessels of the spermatic cord with a 7.5-MHz transducer, but most ultrasound machines in developing countries are equipped with 3.5-MHz transducers. To assess the potential for ultrasound as a tool for diagnosis and epidemiologic assessment in lymphatic filariasis, we compared the performance of 3.5-MHz and 7.5-MHz transducers in 61 men in Recife, Brazil. All men had three ultrasound examinations using a 3.5 MHz transducer and an examination with a 7.5-MHz probe. Using the 7.5-MHz transducer, adult W. bancrofti were detected in 41 men; 81 adult worm nests were detected. Sixty-four (79%) nests were detected with the 3.5-MHz probe, each on all three examinations. The 3.5-MHz probe correctly identified 35 (85.4%) of 41 men as infected; sensitivity increased with lymphatic vessel diameter. Ultrasonographic examination with a 3.5-MHz transducer is a sensitive method for detection of adult W. bancrofti in men and merits consideration as a tool for rapid epidemiologic assessment.

With an estimated 120 million infected persons, lymphatic filariasis is an important public health problem in tropical areas of the world. An estimated 90% of these infections are caused by the parasitic worm Wuchereria bancrofti. Although many infected persons are asymptomatic, an estimated 40 million have filaria-associated lymphedema or testicular hydrocele. The medical, social, and economic costs of these sequelae are enormous; lymphatic filariasis is considered the second leading cause of disability worldwide.

Ultrasound is increasingly used for diagnosis and clinical studies of parasitic diseases such as schistosomiasis and echinococcosis, and recently it has been shown to be a powerful tool for the study of lymphatic filariasis. In 1994, Amaral and others first reported using ultrasound to visualize the spermatic cord. They described a continuous, distinctive, and specific pattern of worm movement called the filaria dance sign. When surgery was performed on patients who exhibited this sign, nests of adult W. bancrofti were found in the lymphatic vessels of the spermatic cord. Extensive clinical and surgical experience has confirmed these initial findings (Dreyer G, unpublished data). and ultrasonographic examinations of men from nonendemic areas have failed to detect the filaria dance sign. The location of the adult worm nests within the lymphatic vessels remains remarkably stable with time. Detection of the filaria dance sign subsequently has been used to diagnose W. bancrofti infection in both men and women and to directly assess the efficacy of antifilarial drugs against the adult worm.

 Virtually all ultrasound studies of lymphatic filariasis have used 5.0- or 7.5-MHz transducers, which provide good resolution of the superficial and dilated lymphatic vessels of the spermatic cord. With a 7.5-MHz transducer, the filaria dance sign can be detected in the scrotal area of at least 80% of men with microfilaremia (Dreyer G, unpublished data) and in a significant proportion of infected men who are microfilaria-negative in 1 ml of filtered blood. However, the need for a 7.5-MHz transducer greatly limits the use of ultrasound for diagnosis and study of lymphatic filariasis. Ultrasound machines for obstetrical use are available in many hospitals and clinics in developing countries, but these machines are usually equipped with 3.5-MHz transducers. The 3.5-MHz transducers generally produce greater tissue penetration and are useful for visualizing internal organs, but they are less suitable for examination of superficial structures. Because the filaria dance sign is so characteristic, we wished to assess the potential for using a 3.5-MHz transducer for field diagnosis and screening for lymphatic filariasis. We compared the performance of ultrasound with 3.5- and 7.5-MHz transducers to detect the filaria dance sign in the scrotal area of infected men.

MATERIALS AND METHODS

The study protocol was approved by the Ethical Review Board of Hospital das Clinicas, Universidade Federal de Pernambuco (Recife, Brazil). Sixty-one men, all asymptomatic long-term residents of greater Recife, were enrolled in the study after written informed consent was obtained. Before the study began, all patients had 1 ml of blood collected between 11:00 PM and 1:00 AM; the blood was passed through a 3-µm Nuclepore filter (Nuclepore, Pleasanton, CA) and examined for microfilariae. Men who were microfilaria-negative in 1 ml of blood had additional specimens of 5 ml and 10 ml collected on subsequent weeks before being considered microfilaria-negative. Patients were unaware of the results of their blood test or ultrasound examinations until the end of the study. Men who were microfilaria-positive and those who had adult W. bancrofti detected on ultrasound examination were treated at the end of the study with diethylcarbamazine or ivermectin.

All 61 men underwent ultrasonographic examinations of the scrotal area by one of two investigators using a Pio Medical Scanner 200 ultrasound machine with a 7.5-MHz transducer. The location of the adult worm nests and the diameter of the lymphatic vessels at the site of the adult worm were recorded. A third investigator, who was blinded to the patient’s microfilaria status and ultrasound results, independently examined the scrotal area of each patient using a Pio Medical portable ultrasound machine with a 3.5-MHz probe. Three such examinations were performed, once a week for three weeks. At the end of the three-week study, the senior
Characteristics and ultrasonographic findings for 41 men with a filaria dance sign (FDS) detectable by ultrasound using a 7.5-MHz transducer and 20 men with no detectable FDS in Recife, Brazil

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>FDS-positive</th>
<th>FDS-negative</th>
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<tbody>
<tr>
<td>Number of men</td>
<td>41</td>
<td>20</td>
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<tr>
<td>Mean age (range), years</td>
<td>21.1 (17–34)</td>
<td>20.5 (18–30)</td>
</tr>
<tr>
<td>No. (%) of men microfilaria-positive*</td>
<td>39 (95.1)</td>
<td>0*</td>
</tr>
<tr>
<td>Geometric mean microfilarial density (range)</td>
<td>144 (1–4,030)</td>
<td>0</td>
</tr>
<tr>
<td>No. (%) FDS-positive with a 3.5-MHz probe</td>
<td>35 (85.4)</td>
<td>0</td>
</tr>
<tr>
<td>No. of adult worm nests (total, median, range)</td>
<td>81, 2, 1–5</td>
<td>0</td>
</tr>
<tr>
<td>No. (%) detected by a 3.5-MHz probe</td>
<td>64 (79)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Men were not considered to be microfilaria-negative until a total of 16 ml of blood, collected between 11:00 pm and 1:00 am, had been filtered and examined microscopically for microfilaria.

Statistical methods. The chi-square test was used to determine the significance of differences between proportions and the Wilcoxon rank sum test was used to compare continuous variables (vessel diameter, age, and geometric mean microfilarial density). Statistical tests involving geometric mean microfilarial density were based on log-transformed values. Linear regression and analysis of variance were used to assess the linear relationship between log microfilarial density and number of adult worm nests.

RESULTS

Using a 7.5 MHz transducer, the filaria dance sign was detected in the lymphatic vessels of the spermatic cord in 41 (67.2%) of 61 men. Mean age was similar for men with and without a detectable filaria dance sign (Table 1). Of the 41 men who had a detectable filaria dance sign, 39 (95.1%) were microfilaria-positive, with a geometric mean microfilarial density of 144/ml of blood. A total of 81 adult worm nests were detected in these 41 men (median = 2, range = 1–5) (Figure 1). Of the 81 nests, 33 (40.7%) were detected in the left hemiscrotum and 48 (59.3%) were found in the right hemiscrotum; 17 (41.5%) men had nests detected bilaterally. Geometric mean microfilarial density was higher in men with bilateral filaria dance signs (338/ml) than in men with unilateral involvement (70/ml; P = 0.05). The number of adult worm nests in each man was positively associated with log microfilarial density (R² = 0.19, P = 0.004).

In relation to the testicle, 24 (29.6%) adult worm nests were supratesticular, 14 (17.3%) were infratesticular, and 43 (53.1%) were paratesticular. The diameter of the lymphatic vessels ranged from 1.2 to 12.6 mm (mean = 3.8 mm) at the site of the adult worm nests. Mean vessel diameter at the site of nests in the supratesticular area was 4.5 mm, compared with 3.5 mm and 3.3 mm in the paratesticular and infratesticular areas, respectively (P = 0.03). Lymphatic vessel diameter was not associated with the number of adult worm nests or with microfilarial density.

Sixty-four (79%) of 81 nests were detected using the 3.5-MHz probe (Figure 2). All of these nests were detected within 30–60 sec on each of the three examinations. Detection of the filaria dance sign was influenced by the diameter of the lymphatic vessel (as measured with the 7.5-MHz probe) and the location of the adult worm nest. At the site of nests that were detected with the 3.5-MHz probe, the mean diameter of the lymphatic vessels was 4.3 mm (range = 2.2–12.6), compared with 1.7 mm (range = 1.2–2.7) for nests that were not detected with this probe (P = 0.0001). Vessel diameter strongly influenced the sensitivity of ultrasound; the 3.5-MHz transducer detected all 54 nests > 2.7 mm in diameter, 10 (76.9%) of 13 nests with vessel diameters 2.2–2.7 mm, and none of 14 nests < 2.2 mm in diameter (P < 0.00001). Sensitivity was also influenced by location of the adult worm nest. Using the 3.5-MHz transducer, we were
able to detect all 24 supratesticular nests, 12 (85.7%) of 14 infratesticular nests, and 28 (65.1%) of 43 paratesticular
nests ($P = 0.003$). Within lymphatic vessels of similar di-
neter, however, this relationship between nest location and
sensitivity of the 3.5-MHz probe was no longer statistically
significant. Sensitivity of the 3.5-MHz transducer was not
influenced by patient age or microfilarial density, or by
whether the nest was located in the left or right hemiscrotum.
None of the 20 men who initially tested negative for the
filaria dance sign using the 7.5-MHz transducer tested pos-
tive with the 3.5-MHz probe on any of the three examina-
tions (100% specificity).

Of 41 men with a detectable filaria dance sign using the
7.5-MHz transducer, 35 (85.4%) were found to have one or
more adult worm nests on examination with the 3.5-MHz
probe. In 25 of these men, the 3.5-MHz probe detected all
the nests that had been observed using the 7.5-MHz probe;
in the remaining 10 men, some, but not all nests (50–67%)
were detected. The sensitivity of the 3.5-MHz transducer
was not influenced by the number of nests detected with the
7.5-MHz transducer.

**DISCUSSION**

Ultrasound examination of the scrotal area using a 7.5-
MHz transducer has been shown to be useful for diagnosis of
*W. bancrofti* infection and for assessing the efficacy of
antifilarial drugs against the adult worm.9,10,15,17,18 The sen-
sitivity of the 7.5-MHz transducer is quite high. Although
one study suggested that the filaria dance sign could be de-
tected in as few as 80% of men with *W. bancrofti* microfi-
lariaemia,13 subsequent studies have demonstrated that ultra-
sound may be more sensitive than detection of either micro-
filaria or circulating filarial antigen in peripheral blood, par-
ticularly when microfilarial density is low (Dreyer G,
unpublished data). For example, in a study by Rocha and
others, 10 (14%) of 73 men who were microfilaria-negative in
16 ml of filtered blood had *W. bancrofti* detected on ul-
trasound; only six of these men had detectable levels of cir-
culating filarial antigen (Og4C3).11

The current study indicates that a 3.5-MHz probe can also
be used to detect the filaria dance sign of *W. bancrofti* with
a relatively high sensitivity (79% of adult worm nests and
85% of infected men) and specificity (100%). The sensitivity
of the 3.5-MHz and 7.5-MHz transducers was equivalent
when the diameter of the lymphatic vessel was greater than
2.7 mm. Detection of the filaria dance sign with the 3.5-
MHz transducer was unreliable when the lymphatic vessel
diameter was less than 2.7 mm. Thus, for practical purposes,
the limit of detection for the 3.5-MHz probe was reached at
a vessel diameter of 2.7 mm. For the 7.5-MHz probe, the
limit of detection appears to be at a vessel diameter of ap-
proximately 1 mm.12 Thus, when maximum resolution of the
ultrasound image is required, such as in studies of drug ef-
ficacy, the 7.5-MHz transducer should continue to be used.

The sensitivity of ultrasound with a 3.5-MHz transducer
compares favorably with that of examination of capillary
blood for microfilaria, particularly in persons with low mi-
icrofilarial densities. Examination of 20–60 μl of capillary
blood (standard volumes for epidemiologic surveys) detects
as few as 26–52% of infected persons who have microfilarial
densities of 1–30/ml in venous blood.20 In contrast, among
eight men in this study who had microfilarial densities ≤ 30/
ml venous blood, ultrasound examination with the 3.5-MHz
probe correctly identified six (75%) as infected.

These findings have important implications for the use of
ultrasound as a tool for rapid epidemiologic assessment of
lymphatic filariasis. In light of recent findings that single-
dose ivermectin or diethylcarbamazine can profoundly sup-
press *W. bancrofti* microfilaraemia for periods of 6–24
months,1 the International Task Force for Disease Eradica-
tion has classified lymphatic filariasis as a potentially eradic-
able disease.21 As in the eradication programs for smallpox
and dracunculiasis,22 epidemiologic techniques must be de-
veloped to rapidly identify communities with active trans-
mission of *W. bancrofti* so that appropriate interventions can
be focused on these communities.23,24

Ultrasound has several features that make it attractive as
a rapid assessment tool, including acceptability, conve-
nience, and sensitivity. Patient acceptance of ultrasound in
studies of lymphatic filariasis has been high, not only in
Brazil, but in India and Haiti, where transducers of 5.0 MHz
or greater have been used to visualize adult *W. bancrofti*
(Kumaraswami V, unpublished data and Streit T, unpublished
data). Ultrasound units equipped with 3.5-MHz transducers
are increasingly available for obstetrics, and portable ma-
.chines are now made for field use.7

The microfilaria of *W. bancrofti* are generally nocturnally
periodic; i.e., they appear in highest concentrations in the
peripheral blood late at night, during the period of most in-
tense biting of the mosquito vector.20 Therefore, detection of
microfilariae in the blood, which is the current standard for
diagnosis, must be done at night. This nocturnal periodicity
has created considerable difficulties for epidemiologic stud-
ies of bancroftian filariasis. Because the ultrasound image
of adult *W. bancrofti* (the filaria dance sign) is remarkably sta-
ble with time, one advantage of ultrasound examination,
which is shared with the recently developed antigen detec-
tion assays,25,26 is that it can be done at any time of the day
or night.

For rapid epidemiologic assessment, it may be possible to
add ultrasound to physical examinations required for certain
types of employment, such as the military. Indeed, in some
areas where filariasis is endemic, hydrocele is a basis for
exclusion from military service. In these settings, ultrason-
ographic screening and data on residential location could be
used to identify areas with transmission of *W. bancrofti*. Ul-
trasound may also prove useful as one of several tools to
certify that lymphatic filariasis has been eliminated from
specified areas. In addition to detection of the adult worm,
ultrasound examination of the scrotal area can detect sub-
clinical morbidity associated with filarial infection, including
lymphangiectasis and hydrocele (Figure 3).13 Because ly-
mphatic vessel diameter tended to be greater at the site of adult
worms in supratesticular locations in the present study, in-
vestigators involved in rapid assessment with a 3.5-MHz
transducer may wish to focus on these locations if exami-
nation time is limited.

Potential disadvantages of ultrasound for rapid epidemi-
ologic assessment include a lack of ultrasound machines in
some areas, a current shortage in developing countries of
persons with training in ultrasonography or in detection of
the filaria dance sign, and a sensitivity that, although comparable to other methods, is less than 100%. In addition, the precise relationship between ultrasonographic detection of adult *W. bancrofti* in men and the level of filarial transmission in the community from which they come is currently unknown. At present, ultrasound is useful as a diagnostic tool primarily in adult men. Based on clinical,27–30 pathologic,29–31 and ultrasound,13 evidence, the scrotal area appears to be a preferred site of adult *W. bancrofti* in men. Although the filaria dance sign has been detected in the breast of an infected woman,18 the preferred locations of adult *W. bancrofti* in women and children remain a mystery. These facts should not diminish the potential usefulness of ultrasound as a tool for rapid epidemiologic assessment, the goal of which should not diminish the potential usefulness of ultrasound as a tool for rapid epidemiologic assessment, the goal of which is to identify affected communities. Indeed, physical examinations of men for hydrocele have already been recommended as rapid assessment techniques. This indicator appears to correlate well with the prevalence of *W. bancrofti* microfilaremia in the community, even though only half the population is eligible for such screening.23

In an earlier study, we found that microfilarial density was higher in men with bilateral filarial dance signs than in men with unilateral involvement.13 The results of the current study confirm these earlier findings and demonstrate further that microfilarial density is significantly associated with the number of adult worm nests detected by ultrasound.

In summary, ultrasonographic examination with a 3.5-MHz transducer appears to be both sensitive and specific for adult *W. bancrofti* in the scrotal area of infected men. The use of ultrasound for rapid epidemiologic assessment merits further consideration.

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Authors’ addresses: Gerusa Dreyer and Abiel Santos, Departamento de Parasitologia, Centro de Pesquisas Aggeu Magalhaes-FIOCRUZ, Av. Moraes Rego S/N Cidade Universitaria, CEP 50670-420, Recife PE, Brazil. Joaquim Noroés, Hospital das Clinicas, Universidade Federal de Pernambuco, Recife, Brazil. Fernando Amaral, Medias, Memorial Imagem e Diagnostico, Recife, Brazil. David Addiss, Division of Parasitic Diseases, Mailstop F-22, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, Chamblee, GA 30341-3724.

Reprint requests: Gerusa Dreyer, Departamento de Parasitologia, Centro de Pesquisas Aggeu Magalhaes-FIOCRUZ, Av. Moraes Rego s/n Cidade Universitaria, CEP 50670-420, Recife, PE, Brazil.

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