Short Report: Semi-Quantitative Scoring of an Immunochromatographic Test for Circulating Filarial Antigen

Cédric B. Chesnais,* François Missamou, Sébastien D. S. Pion, Jean Bopda, Frédéric Louya, Andrew C. Majewski, Gary J. Weil, and Michel Boussinesq

Unité Mixte Internationale 233, Institut de Recherche pour le Développement and University of Montpellier 1, Montpellier, France;
Programme National de Lutte contre l'Onchocercose, Ministère de la Santé et de la Population, Brazzaville, Republic of Congo;
Filariasis and other Tropical Diseases Research Centre, Yaoundé, Cameroon; Infectious Diseases Division,
Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri

Abstract. The value of a semi-quantitative scoring of the filarial antigen test (Binax Now Filariasis card test, ICT) results was evaluated during a field survey in the Republic of Congo. One hundred and thirty-four (134) of 774 tests (17.3%) were clearly positive and were scored 1, 2, or 3; and 11 (1.4%) had questionable results. *Wuchereria bancrofti* microfilariae (mf) were detected in 41 of those 133 individuals with an ICT test score ≥ 1 who also had a night blood smear; none of the 11 individuals with questionable ICT results harbored night mf. Cuzick's test showed a significant trend for higher microfilarial densities in groups with higher ICT scores ($P < 0.001$). The ICT scores were also significantly correlated with blood mf counts. Because filarial antigen levels provide an indication of adult worm infection intensity, our results suggest that semi-quantitative reading of the ICT may be useful for grading the intensity of filarial infections in individuals and populations.

The current methods of choice for diagnosis of active infections with *Wuchereria bancrofti*, the main agent of lymphatic filariasis (LF), are based on detection of microfilaria (mf) or circulating filarial antigens (CFA) in blood. Although antigenemia can be detected by enzyme-linked immunosorbent assay (ELISA), field studies and filariasis elimination programs generally use a point-of-care immunochromatographic card test (ICT) (Binax Now Filariasis ICT test, Alere, Portland, ME).¹

Several publications have addressed the issue of the choice of diagnostic tools for use in different phases of LF control programs.^{2–4} Before the initiation of mass drug administration (MDA), the simplest method for assessing LF endemicity in an area is to measure the proportion of individuals with CFA, using the ICT test. Night blood testing of persons with positive ICT tests can be used to estimate the mf rate in the population. After MDA has been initiated, antigen testing can also be used to assess the impact of MDA.⁴ However, as the primary objective of the control programs is to remove mf (required for transmission of the parasite), and as filarial antigen rates decrease more slowly than the microfilaria rates,⁵ it is also useful to follow mf rates during MDA programs.⁶

Another key factor determining the duration of the control programs is the impact of MDA on the lifespan of adult *W. bancrofti*.^{7,8} Two methods have been used to evaluate this, namely ultrasonography, which permits visualization of motile adult worms (the “filarial dance sign”) in some infected individuals, and quantitative CFA testing.^{9–13} However, ultrasonography for LF is not sensitive for diagnosis of filarial infections and it requires expensive equipment and highly trained personnel. Quantitative CFA testing also requires highly trained personnel and significant laboratory infrastructure. Thus, these techniques are not feasible for routine use in LF control programs. Therefore, this study was designed to explore the value of using a scoring system for ICT tests as a semi-quantitative method

for assessing *W. bancrofti* adult worm loads in individuals and in populations.

An LF survey was conducted in October 2012 in the village of Séké Pembé, located in the Bouenza division of the Republic of Congo. Séké Pembé had not received MDA for LF, but some village residents had received treatment with mebendazole for intestinal worm infections. A detailed census of the population had been performed 1 month before the LF survey, and all persons 5 years of age and above were invited to be tested for *W. bancrofti* infection. Blood was collected by finger prick, and ICT tests were performed according to the manufacturer's instructions. The ICT tests were read in 10 minutes by a single trained operator (author MB). Intensity of the test (“T”) line was scored as follows: (0) indicating no visible “T” lines; (0.5) for questionable “T” lines or very faint shadows requiring the opinion of a second examiner; (1) for clearly visible “T” lines but weaker than the control line; (2) for “T” lines that were approximately as dark as the control line; and (3) for cards with a “T” line darker than the control line.

Subjects with ICT scores > 0 were called back for a second blood sampling (thick smear for detection of mf) between the hours of 10:00 PM and 1:00 AM. Blood was collected using a capillary tube, and two blood smears (volume 70 μ L) were prepared for each subject. On the next day, the blood smears were dehemoglobinized, stained with Giemsa, and read by two experienced microscopists (authors JB and MB). As loiasis and *Mansonella perstans* filariasis are also endemic in the area, special attention was paid to identify the species of each mf present in the thick smears, and counts were recorded separately for each species. The mf density was defined as the highest count of the two slides and expressed as mf per 70 microliters of blood (mf/70 μ L). The maximum ratio between the higher and lower mf count from two slides from individuals was 2.9 (22 and 64 mf/70 μ L). The median ratio was 1.5 (interquartile range [IQR]: 1.2–1.7). When not specified, all results presented below will refer only to *W. bancrofti* mf.

The distribution of the ICT scores was compared between microfilaremic and amicrofilaremic subjects using the Wilcoxon test. Mean microfilarial densities were compared between ICT score groups using the Cuzick's test for trend¹⁴; the correlation

* Address correspondence to Cédric B. Chesnais, Institut de Recherche pour le Développement (UMI 233), 911 avenue Agropolis, BP 64501, 34394 Montpellier cedex 5, France. E-mail: cedric.chesnais@ird.fr

between ICT scores and mf density was assessed using the Spearman's rank correlation coefficient. All analyses were performed using STATA 12.1 (StataCorp, College Station, TX).

This study was approved by the ethical committee of the Republic of Congo (Ministry of Public Health). Written informed consent was obtained from all adults participating in the study and from parents or legal guardians of minors.

The ICT tests were performed on 774 of the 876 individuals ≥ 5 years of age and recorded during the preliminary census. A total of 145 individuals had an ICT score > 0 ; 11 had scores of 0.5 (1.4% of the population tested), 85 had scores of 1 (11%), 35 had scores of 2 (4.5%), and 14 had scores of 3 (1.8%).

All but one of the individuals with ICT scores > 0 had night blood collected for mf testing. Only 3 and 2 patients had *Loa loa* or *M. perstans* mf in the blood smears, respectively. *Wuchereria bancrofti* microfilaremia was detected in 41 individuals. This number represented 5.3% (41 of 773) of the population tested, 28.5% (41 of 144) of those ICT scores > 0 , and 30.8% (41 of 133) of those with ICT scores > 0.5 . None of the individuals with ICT of 0.5 was microfilaremic. The proportions of people with microfilaremia were 10.7%, 57.1%, and 85.7% in people with ICT scores of 1, 2, and 3, respectively. The distribution of the ICT scores among the microfilaremic and amicrofilaremic ICT positive subjects is presented in Figure 1; the median score was significantly higher in microfilaremic than in individuals with no mf (Wilcoxon test; $P < 0.001$).

The arithmetic mean mf density in those who were microfilaremic was 44.1 mf/70 μ L. Figure 2 shows the distributions of the microfilarial densities for each ICT score group. The overall (i.e., including amicrofilaremic individuals) mean mf densities in score groups 1, 2, and 3 were 1.6 (SD: 5.2), 22.2 (SD: 41.7), and 64.6 mf/70 μ L (SD: 87.2), respectively (Figure 2, right side). The mean mf densities among microfilaremic individuals were 14.1 (SD: 8.1) in group 1, 38.8 (SD: 49.3) in group 2, and 75.4 mf/70 μ L (SD: 90.0) in group 3 (Figure 2, left side). Cuzick's test showed a highly significant trend for higher microfilarial densities in groups with higher ICT scores ($P < 0.001$ for the entire study population and $P = 0.021$ in those

FIGURE 1. Frequency distributions of the immunochromatographic card test (ICT) scores in microfilaremic (striped boxes) and amicrofilaremic (white boxes) subjects with positive ICT test results.

FIGURE 2. Distribution of *Wuchereria bancrofti* microfilarial densities by immunochromatographic card test (ICT) score for microfilaremic subjects (left panel) and for all ICT positive subjects (right panel). Horizontal lines represent arithmetic means and 95% confidence intervals.

who were microfilaremic). The correlation, at the individual level, between ICT scores (for those with scores ≥ 1) and mf density was positive and significant ($\rho = 0.61$, $P < 0.001$ for all subjects with scores ≥ 1 ; and $\rho = 0.37$, $P = 0.0187$ for subjects with microfilaremia).

Circulating filarial antigen levels are believed to be correlated with adult worm counts in humans with bancroftian filariasis.^{1,9} Though it is impossible to accurately determine the number of macrofilariae *in vivo*, this assertion is supported by studies that have shown a relationship between antigen levels and microfilarial densities in mf-positive individuals before treatment,^{1,15-22} and a relationship between antigen levels and microfilarial densities with the frequency of detection of worm nests by ultrasonography.^{10-12,16,23}

The original AD12 ELISA and the Og4C3-based TropBio ELISA tests are useful for quantitating filarial antigenemia.^{1,9,24} However, these tests require highly trained personnel and significant laboratory infrastructure, and they are usually not practical for use by national LF elimination programs.¹³ The fact that the ICT test can provide a semi-quantitative assessment of antigen levels (and perhaps relative adult worm burdens) may be helpful to LF elimination programs as an indicator of infection intensity in populations. Similarly, changes in mean ICT scores could provide an indication of the impact of MDA on adult worms in endemic communities. Decreases in mean ICT scores among those with positive antigen tests after the first rounds of MDA might provide a more accurate indication of the success of MDA than changes in antigen prevalence rates.

Our study also provided new data on the issue of equivocal ICT card test results. Because all 11 of the people with ICT scores of 0.5 (very faint shadow lines) were amicrofilaremic, our study suggests that cards with very faint shadow lines should be considered to be negative for filarial antigenemia.

The ICT filarial antigen tests are widely performed before, during, and after MDA programs. Our results suggest that it is useful to score these tests and that test scores can be used together with antigen prevalence data. A recently published study used a similar scoring system to evaluate the Alere Filariasis Test Strip that is expected to replace the ICT test

in the near future.²⁵ Although the Test Strip was more sensitive than the ICT test in that study, antigen scores for blood samples that were positive by either test were highly correlated. Note that there is no additional cost associated with scoring filarial antigen tests, and scores can be written directly on the cards. Antigen test score data from other endemic areas with different infection rates and treatment histories will help to confirm the practical value of antigen test scoring for LF elimination programs.

Received May 8, 2013. Accepted for publication June 30, 2013.

Published online September 9, 2013.

Acknowledgments: We thank the residents of Séké Pembé (especially the village chiefs and other leaders) for their active participation in the study. We also thank Ministry of Health staff who participated in the study (Maurice Mpompolo and Dieudonné Boungou; Albertine Mpika; and Valentin Angoni, Clément Biyoukala, Gyslain Boukete, Daniel Mavoungou Dibamba, Célestin Ibouanga, Sébastien Lemboumi, Anicet Madoulou, César Manene, Jean Ngoma, Magloire Ntama, and Gilbert Nzaou).

Financial support: This research was funded by the Bill & Melinda Gates Foundation.

Disclaimer: The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation.

Authors' addresses: Cédric B. Chesnais, Sébastien D. S. Pion, and Michel Boussinesq, Institut de Recherche pour le Développement (UMI 233), Montpellier Cedex 5, France, E-mails: cedric.chesnais@ird.fr, sebastien.pion@ird.fr, and michel.boussinesq@ird.fr. François Missamou and Frédéric Louya, Programme National de Lutte contre l'Onchocercose, Direction de l'Epidémiologie et de la Lutte contre la Maladie, Ministère de la Santé et de la Population, Brazzaville, Republic of Congo, E-mails: missamou_franco@yahoo.com and fredericlouya@yahoo.fr. Jean Bopda, Filariasis and Other Tropical Diseases Research Centre, Yaoundé, Cameroon, E-mail: f_bopda@yahoo.fr. Andrew C. Majewski and Gary J. Weil, Infectious Diseases Division, Washington University School of Medicine, St. Louis, MO, E-mails: amajewsk@dom.wustl.edu and gweil@dom.wustl.edu.

Reprint requests: Cédric B. Chesnais, Institut de Recherche pour le Développement (UMI 233), 911 avenue Agropolis, BP 64501, 34394 Montpellier cedex 5, France, Tel: 33-4-67-41-61-52, E-mail: cedric.chesnais@ird.fr.

REFERENCES

- Weil GJ, Jain DC, Santhanam S, Malhotra A, Kumar H, Sethumadhavan KV, Liftis F, Ghosh TK, 1987. A monoclonal antibody-based enzyme immunoassay for detecting parasite antigenemia in bancroftian filariasis. *J Infect Dis* 156: 350–355.
- Gass K, Beau de Rochars MV, Boakye D, Bradley M, Fischer PU, Gyapong J, Itoh M, Ituaso-Conway N, Joseph H, Kyelem D, Laney SJ, Legrand A-M, Liyanage TS, Melrose W, Mohammed K, Pilote N, Ottesen EA, Plichart C, Ramaiah K, Rao RU, Talbot J, Weil GJ, Williams SA, Won KY, Lammie P, 2012. A multicenter evaluation of diagnostic tools to define end-points for programs to eliminate bancroftian filariasis. *PLoS Negl Trop Dis* 6: e1479.
- Lammie PJ, 2004. Research directly linked with GPELF activities (operational research): essential tools–diagnostics. *Am J Trop Med Hyg* 71 (Suppl): 3–6.
- Weil GJ, Ramzy RM, 2007. Diagnostic tools for filariasis elimination programs. *Trends Parasitol* 23: 78–82.
- Schuetz A, Addiss DG, Eberhard ML, Lammie PJ, 2000. Evaluation of the whole blood filariasis ICT test for short-term monitoring after antifilarial treatment. *Am J Trop Med Hyg* 62: 502–503.
- World Health Organization, 2011. *Monitoring and Epidemiological Assessment of Mass Drug Administration in the Global Programme to Eliminate Lymphatic Filariasis: A Manual for National Elimination Programmes*. Available at: http://whqlibdoc.who.int/publications/2011/9789241501484_eng.pdf. Accessed March 26, 2013.
- Vanamail P, Subramanian S, Rajagopalan PK, 1990. A mathematical analysis of various factors involved in transmission of bancroftian filariasis in Pondicherry. *Indian J Med Res* 91: 289–292.
- Dreyer G, Addiss D, Norões J, 2005. Does longevity of adult *Wuchereria bancrofti* increase with decreasing intensity of parasite transmission? Insights from clinical observations. *Trans R Soc Trop Med Hyg* 99: 883–892.
- Chanteau S, Mouliat-Pelat JP, Glaziou P, Nguyen NL, Luquaud P, Plichart C, Martin PM, Cartel JL, 1994. Og4C3 circulating antigen: a marker of infection and adult worm burden in *Wuchereria bancrofti* filariasis. *J Infect Dis* 170: 247–250.
- Simonsen PE, Bernhard P, Jaoko WG, Meyrowitsch DW, Malecela-Lazaro MN, Magnussen P, Michael E, 2002. Filariasis dance sign and subclinical hydrocoele in two East African communities with bancroftian filariasis. *Trans R Soc Trop Med Hyg* 96: 649–653.
- Amaral F, Dreyer G, Figueredo-Silva J, Noroes J, Cavalcanti A, Samico SC, Santos A, Coutinho A, 1994. Live adult worms detected by ultrasonography in human Bancroftian filariasis. *Am J Trop Med Hyg* 50: 753–757.
- Noroes J, Addiss D, Amaral F, Coutinho A, 1996. Occurrence of living adult *Wuchereria bancrofti* in the scrotal area of men with microfilariemia. *Trans R Soc Trop Med Hyg* 90: 55–56.
- Walther M, Muller R, 2003. Diagnosis of human filariases (except onchocerciasis). *Adv Parasitol* 53: 149–193.
- Cuzick J, 1985. A Wilcoxon-type test for trend. *Stat Med* 4: 87–90.
- Ramzy RM, Gad AM, Faris R, Weil GJ, 1991. Evaluation of a monoclonal-antibody based antigen assay for diagnosis of *Wuchereria bancrofti* infection in Egypt. *Am J Trop Med Hyg* 44: 691–695.
- Rocha A, Braga C, Belém M, Carrera A, Aguiar-Santos A, Oliveira P, Texeira MJ, Furtado A, 2009. Comparison of tests for the detection of circulating filarial antigen (Og4C3-ELISA and AD12-ICT) and ultrasound in diagnosis of lymphatic filariasis in individuals with microfilariae. *Mem Inst Oswaldo Cruz* 104: 621–625.
- Simonsen PE, Dunyo SK, 1999. Comparative evaluation of three new tools for diagnosis of bancroftian filariasis based on detection of specific circulating antigens. *Trans R Soc Trop Med Hyg* 93: 278–282.
- El-Moamly AA, El-Sweify MA, Hafez MA, 2012. Using the AD12-ICT rapid-format test to detect *Wuchereria bancrofti* circulating antigens in comparison to Og4C3-ELISA and nucleopore membrane filtration and microscopy techniques. *Parasitol Res* 111: 1379–1383.
- Rocha A, Addiss D, Ribeiro ME, Noroes J, Baliza M, Medeiros Z, Dreyer G, 1996. Evaluation of the Og4C3 ELISA in *Wuchereria bancrofti* infection: infected persons with undetectable or ultra-low microfilarial densities. *Trop Med Int Health* 1: 859–864.
- Lalitha P, Ravichandran M, Suba S, 1998. Quantitative assessment of circulating antigens in human lymphatic filariasis: a field evaluation of monoclonal antibody-based ELISA using blood collected on filter strips. *Trop Med Int Health* 3: 41–45.
- Itoh M, Weerasooriya MV, Gunawardena NK, Mudalige MP, Samarawickrema WA, Kimura E, 1999. *Wuchereria bancrofti* antigenemia in Sri Lanka. *Trop Med Int Health* 4: 207–210.
- Tisch DJ, Hazlett FE, Kastens W, Alpers MP, Bockarie MJ, Kazura JW, 2001. Ecologic and biologic determinants of filarial antigenemia in bancroftian filariasis in Papua New Guinea. *J Infect Dis* 184: 898–904.
- Britto L, Ravichandran V, Das LK, Pani SP, 2009. Implications of 2D ultrasound findings of the scrotum in asymptomatic microfilaria carriers of *Wuchereria bancrofti* infection. *Trop Med Health* 37: 21–25.
- More SJ, Copeman DB, 1990. A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. *Trop Med Parasitol* 41: 403–406.
- Weil GJ, Curtis KC, Fakoli L, Fischer K, Gankpala L, Lammie PJ, Majewski AC, Won KY, Bolay FK, Fischer PK, 2013. Laboratory and field evaluation of a new rapid test for detecting *Wuchereria bancrofti* antigen in human blood. *Am J Trop Med Hyg* 89: 11–15.