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# Mass Drug Administration Trial to Eliminate Lymphatic Filariasis in Papua New Guinea: Changes in Microfilaremia, Filarial Antigen, and Bm14 Antibody after Cessation

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*Abstract.* Laboratory tools to monitor infection burden are important to evaluate progress and determine endpoints in programs to eliminate lymphatic filariasis. We evaluated changes in *Wuchereria bancrofti* microfilaria, filarial antigen and Bm14 antibody in individuals who participated in a five-year mass drug administration trial in Papua New Guinea. Comparing values before treatment and one year after four annual treatments, the proportion of microfilaria positive individuals declined to the greatest degree, with less marked change in antibody and antigen rates. Considering children as sentinel groups who reflect recent transmission intensity, children surveyed before the trial were more frequently microfilaria and antibody positive than those examined one year after the trial stopped. In contrast, antigen positive rates were similar in the two groups. All infection indicators continued to decline five years after cessation of mass drug administration; Bm14 antibody persisted in the greatest proportion of individuals. These data suggest that Bm14 antibody may be a sensitive test to monitor continuing transmission during and after mass drug administration aimed at eliminating transmission of lymphatic filariasis.

## INTRODUCTION

Central to the strategy of the global program to control and eliminate lymphatic filariasis (LF) is mass administration of anti-filarial drugs (MDA) to reduce the reservoir of blood microfilaria (MF) below a threshold necessary for continuing transmission by local mosquito vectors.[1,2] The development of sensitive, specific, and standardized laboratory tests to quantify the MF and infection burden at the community level has been a major factor in the design and execution of this effort.[3] The urgency to understand the limitations of these tests and how to interpret changes in them over time is underscored by the remarkable progress of MDA programs to date. As of 2005, the global alliance to eliminate LF estimated that 250 million of the 1106.8 million persons in the world at risk for LF participated in one or more of the recommended four to six annual rounds of MDA, consisting of single-dose diethylcarbamazine combined with albendazole, albendazole combined with ivermectin, or distribution of diethylcarbamazine-medicated table salt (www.filariasis.org).

In the case of *Wuchereria bancrofti* infection, assays that detect circulating Og4C3 antigen and IgG4 antibody to a recombinant LF protein designated Bm14 have been proposed to identify endemic populations where MDA should be implemented and to inform decisions regarding when MDA should be stopped.[1,4,5] The antigen and antibody assays have several advantages over microscopic identification of MF in blood, which is the traditional method of diagnosing LF infection.[3,6,7] They are more sensitive (i.e., MF-negative persons with positive antigen or antibody tests are frequently identified)[8] and both overcome the logistical constraint of obtaining blood at night, which is necessary in the many endemic areas where MF have nocturnal periodicity. The Og4C3 antigen on which the ELISA[9] and diagnostic card test[6] are based is secreted by *W. bancrofti* (but not *Brugia* species) adult worms. Antibodies to Bm14, a recombinant protein identified originally by screening a *B. malayi* cDNA library,[10] may be present in the persons with *W. bancrofti*, *B. malayi,* or *B. timori* infection.[4] Bm14 antibody may detect recent exposure to infective larvae and the presence of adult worms, because animal studies indicate that the antibody appears during the pre-patent period of infection. The antigen and antibody assays are highly specific for LF with minimal to no cross-reactivity for gastrointestinal worms that may coexist in populations where LF is endemic. To advance our understanding of how these tests might be used to interpret the progress and long-term impact of MDA programs, we examined plasma obtained from adults and children who participated in a 5-year MDA trial in Papua New Guinea that compared the efficacy of single-dose diethylcarbamazine alone to diethylcarbamazine combined with ivermectin.

## MATERIALS AND METHODS

**Research participants and ethical approval.** Results of the MDA trial up to 1 year after completion of the fourth of five annual rounds of MDA with single-dose diethylcarbamazine alone versus diethylcarbamazine combined with ivermectin (administered in 1994, 1995, 1996, 1997, and 1998) with respect to changes in MF status, lymphatic pathology, and entomologic measures of transmission have been published.[11,18] MF prevalence and annual transmission potential decreased by 77–86% and 84–97%, respectively; there was not a significant difference between villages randomized to diethylcarbamazine alone versus diethylcarbamazine combined with ivermectin. Greater reduction in transmission was observed in villages where the pre-MDA transmission potential was moderate (24–167 bites from mosquitoes containing infective larvae per person per year) relative to those where pre-MDA transmission was higher (224–742 bites from mosquitoes containing infective larvae per person per year). The MDA trial and informed consent for use of the participants' blood samples for studies described here were reviewed and approved by the Human Investigations Institutional Review

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Board of University Hospitals of Cleveland/Case Western Reserve University, Cleveland, OH, and Medical Research Advisory Committee of the government of Papua New Guinea.

**Measures of infection status.** MF levels were quantified by microscopy after Nuclepore filtration (SPI Supplies, West Chester, PA) of 1 mL venous blood obtained between 10:00 PM and 2:00 AM.[12] MF status was designated as negative or positive without regard to the absolute level of MF. Og3C4 antigen was measured by two-site antigen capture ELISA as described previously.[12] A positive antigen test was scored when the optical density (OD) of the sample was greater than the OD generated by standards indicated by the manufacturer (TropBio, Townsville, Australia) to be in titer group 3 or higher. Bm14-histidine fusion protein and control chloramphenicol acetyl transferase-histidine fusion proteins were provided by the NIH/NIAID Filariasis Research Reagent Repository Center (FR3)–Molecular Resources Division (Smith College, Amherst, MA). ELISA for IgG4 antibody to the Bm14 and control proteins[13] was performed exactly as recommended by instructions from the developers of the assay, Drs Gary Weil (Washington University, St. Louis, MO) and Steve Williams (Smith College, Amherst, MA). Briefly, aliquots of plasma diluted 1:100 were tested in parallel for IgG4 antibody to Bm14 (ORF 459 bp) and control protein. Polyvinyl 96-well microtiter plates (Falcon, Becton-Dickinson Biosciences, San Jose, CA) were coated with Bm14 or control protein (0.2 μg in 100 μL/well) in 0.06 mol/L carbonate buffer, pH 9.6, incubated overnight at 37°C, washed six times with PBS/0.5% Tween 20, blocked with buffer containing 5% heat-inactivated fetal calf serum for 30 min at 37°C, and washed six times. Each assay included plasma from 10 North Americans who had never been exposed to LF (negative controls) and a positive control constituted by pooling plasma from six MF-positive Papua New Guineans (positive control). All samples, controls, and blanks were run in duplicate against each protein. The plates were incubated for 2 hours at 37°C after addition of plasma and washed six times in buffer, and horseradish peroxidase–conjugated mouse monoclonal anti-human IgG4 (Zymed, Invitrogen, Carlsbad, CA) diluted 1:667 in phosphate-buffered saline was added at 100 μL/well. After incubation for 1 h at room temperature, the plates were washed, and *o*-phenylenediamine substrate (Sigma, St. Louis, MO) was added. The reaction was stopped by addition of 50 μL 10% $H_2SO_4$, and $OD_{490}$ was read on an ELISA plate reader. After subtracting the blanked OD from both the Bm14 and control protein-coated wells, net OD values for Bm14 minus control protein wells were determined. Values > 3 SD above the mean of 10 North American negative controls were considered positive. Positive samples were further stratified by the number of SDs above the control mean: +, 3–6 SD; ++, 6–10 SD; +++, 10–50 SD; ++++, > 50 SD. Positive samples described here were +++ to ++++ (data not shown).

**Plasma samples selected for study.** We performed antigen and antibody assays on plasma (corresponding MF values were already known) from three groups of individuals who resided in the communities where the MDA trial was conducted between 1993 and 1999. First, to evaluate the impact of MDA on infection status of persons with long-standing exposure to *W. bancrofti*, we analyzed paired samples obtained from 189 individuals 1 year before the first annual MDA and 1 year after the fourth annual MDA (i.e., 5 years later). Second, to assess the immediate impact of MDA-mediated reduction in transmission on infection markers, MF, antigen, and antibody status of 46 1- to 6-year-old children who resided in the study villages 1 year before the trial began (i.e., preceding the first round of MDA) were compared with those of 71 1- to 6-year-old children 1 year after the fourth round of MDA. Neither group of children was given antifilarial drugs before blood samples were obtained, because age < 5 years was an exclusion criterion at the time the study was done. Third, to evaluate the long-term impact of MDA, we compared the results for all available data for individuals examined pre-MDA, 1 year after the fourth round, and 5 years after the fifth round of MDA. Samples from children residing in villages with high pre-MDA transmission intensity were not available. Comparisons for this part of the study were therefore limited to children residing in moderate transmission villages.

**Statistics.** Three analyses were performed: a "paired" comparison of infection assays during two consecutive observations (pre-MDA versus post-MDA) in the same individuals (Bm14 assay was initially performed only on individuals with complete follow-up); a comparison of infection assays among all available children (pre- versus post-MDA); and an aggregate analysis from a random sample of individuals observed 5 years after treatment was stopped. A McNemar test was used to compare the change in diagnostic assay status among the cohort of individuals observed both pre-MDA and 1 year after the final MDA. $\chi^2$ statistics were used to compare diagnostic assay status between time points for the non-paired analyses. Antigen level was characterized according to a standardized scale of arbitrary Og4C3 antigen units, as described by the manufacturer (TropBio, Townsville, Australia) and in our earlier work.[12] Comparisons of antigen levels were made using the Mann-Whitney *U* test statistic. $P < 0.05$ was considered statistically significant. SAS version 9.1 (SAS Institute, Cary, NC) was used for all analyses.

## RESULTS

**Impact of four rounds of MDA on established *W. bancrofti* infection.** One year before commencement of the MDA trial, 70% (133/189), 84% (148/177), and 89% (168/189) of the study participants were MF, antigen, and antibody positive. Five years later, 1 year after the fourth annual MDA, the percentage positive for the respective infection indicators was 4%, 78%, and 49% (Figure 1). The greatest reduction was observed for MF status (98% converted from MF positive to

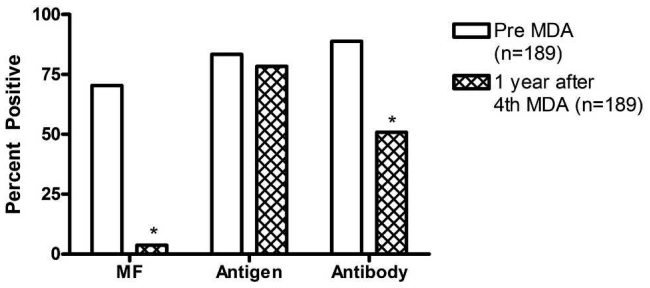


FIGURE 1. Short-term impact of four annual mass drug administrations. The difference in infection assay responses between paired samples before MDA and 1 year after the fourth MDA. *Significant difference ($P < 0.001$) from pre-treatment.

MF negative; $P < 0.001$). Bm14 IgG4 antibodies were lost in 45% of subjects who were positive at the beginning of the study ($P < 0.001$).

Eleven percent of antigen-positive individuals in the moderate transmission villages converted to negative status ($P = 0.002$), but no conversions were observed in the high transmission communities. MF conversions were also slightly higher in the moderate relative to high transmission villages (98.6% versus 90.2%, $P = 0.047$); however, antibody changes were comparable between the two (conversion from positive to negative occurred in 48.1% and 39.1% of residents of moderate versus high transmission; $P = 0.254$). Neither before MDA nor 1 year after the fourth MDA did antigen and antibody status correlate with each other ($\kappa = 0.26$ and 0.17, respectively). No individuals who were MF, antigen, or antibody negative before MDA started (56, 29, and 21 of 189 individuals) were positive 5 years later.

**Comparison of infection indicators in sentinel groups of children examined before the start of MDA with those examined one year after the fourth annual round of MDA.** Children born 1–6 years before MDA began (i.e., those exposed to transmission conditions that prevailed before the MF reservoir was reduced by MDA) had an MF positive prevalence of 30% (13/44). Greater proportions were antigen positive (48%) and antibody positive (78%). In contrast, a group of children 1–6 years of age examined 1 year after the fourth MDA (i.e., those born during the time the trial was being conducted) had significantly lower MF-positive (6%, $P < 0.001$) and antibody-positive rates (37%, $P < 0.001$; Figure 2). Unlike MF and antibody, the proportion of antigen-positive children in the pre-MDA and post-MDA groups was similar.

**Long-term impact of MDA on infection indicators.** Long-term follow-up was limited to communities with pre-treatment annual transmission potential between 24 and 167 bites from infective mosquitoes per person per year. All three infection markers continued to decrease 1 year after the fourth MDA and 5 years after the fifth MDA (Figure 3). The greatest reduction was in MF and antigen rates (98.3% and 72.7%, $P < 0.001$), with less marked decline (51.9%, $P < 0.001$) in the proportion of individuals who were antibody positive. Among these communities with moderate pre-MDA LF transmission, antigen prevalence decreased 29.9% ($P < 0.001$) 1 year after the fourth MDA and 72.7% 5 years after the fifth MDA ($P < 0.001$). Antigen-positive individuals also

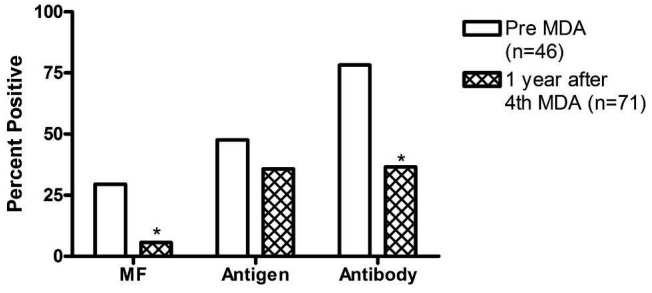


FIGURE 2. Comparison of infection assays on children exposed and not exposed to MDA. Children 1–6 years of age before the start of MDA were compared with children 1–6 years of age 1 year after the fourth MDA was completed. *Significant difference ($P < 0.001$) from pre-treatment.

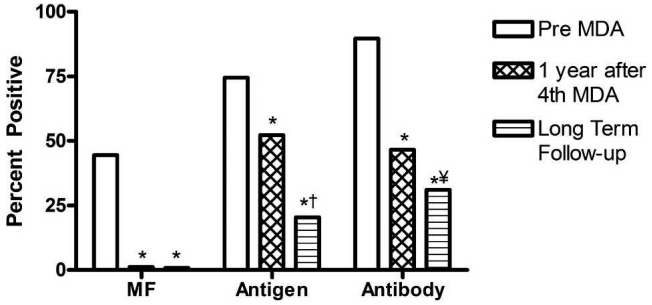


FIGURE 3. Long-term impact of five annual mass drug administrations. Community infection status before MDA and 1 year after the fourth MDA (first two columns of each set) were compared with the same community 5 years after of the fifth MDA (third column of each set). *Significant difference ($P < 0.001$) from pre-treatment; ¥ and †Significant difference from the end of MDA ($P = 0.0086$ and $P < 0.001$, respectively). Sample size varied according to sample and assay availability (sample size by ordered columns: MF, 1,700, 1,389, 535; antigen, 1,105, 1,388, 531; antibody, 116, 116, 161).

experienced significant decreases in the level of antigenemia. The median value of the group decreased from 813 antigen units pre-MDA to 599 units 1 year post-MDA. Five years after the fifth MDA, the median level was 322 antigen units ($P < 0.001$).

**Relation between participation in MDA and changes in LF infection indicators.** To assess whether compliance with MDA affected changes in infection status detected by these monitoring tests, we evaluated the relationship between the number of doses of MDA an individual actually took (consumption of the drugs was directly observed by the investigators) and conversion from positive to negative status in individuals who were eligible to take anti-filarial drugs (i.e. all persons > 5 years old with the exception of pregnant women).

Of the 189 participants observed pre-MDA and 1 year after the fourth annual MDA, 0.5%, 3%, 7%, 27%, and 62% received zero, one, two, three, and four annual doses of MDA. Ninety-one percent to 100% of the pre-MDA MF-positive individuals were MF negative 1 year after the fourth round of MDA. No antigen-positive individuals converted to antigen negative among those who received one to three MDAs; 10 of the 95 antigen positive individuals (10.5%) who received all four MDAs did convert to antigen-negative status 1 year after the fourth MDA. Bm14 antibody converted to negative in 27–50% of antibody-positive individuals independently of the number of annual doses of anti-filarial drugs consumed. A single individual did not receive any MDA. This male adult was MF and antigen negative but Bm14 positive before starting MDA; he remained MF and antigen negative and lost Bm14 antibody at the end of the observation period 5 years later. No individuals converted from a negative to positive result for any of these assays during the study.

The number of doses received by individuals varied during the five annual MDAs. Overall, 1%, 6%, 10%, 14%, 19%, and 50% received zero, one, two, three, four, or five annual doses of MDA, respectively. Only 4 of 535 participants were MF positive 5 years after the final MDA (0.75%), and 3 of these had not received any MDA as a result of their recent immigration into the study villages. Antigen-positive rates for recipients of zero to five doses of MDA ranged from 0% to 50%; antibody-positive rates ranged from 0% to 40%. None

of these rates correlated with the frequency of MDA participation.

## DISCUSSION

Results of this study provide additional insight into the interpretation of the various laboratory tests of human infection status that are currently available to monitor progress of LF elimination programs. The data suggest that, relative to determination of MF by microscopic inspection of blood, the traditional gold standard used to evaluate the MF reservoir, IgG4 antibody to Bm14, is better suited to monitor progress and possibly detect recrudescent or persisting transmission of *W. bancrofti* after MDA has ceased. This contrasts with measurements of filarial Og4C3 antigenemia, which remained positive months to years after administration of anti-filarial drugs. Knowledge of the transmission intensity immediately preceding and at the time blood was obtained for follow-up evaluation of antigen and antibody status will be needed to evaluate more definitively the relative values of these two tests as monitoring tools. An additional limitation of our study is that it did not include the currently recommended MDA regimen of annual single doses of diethylcarbamazine combined with albendazole, which may have a greater effect on eliminating *W. bancrofti* adult worms than diethylcarbamazine alone or diethylcarbamazine combined with ivermectin.[14] We believe, however, that omission of albendazole from this earlier MDA trial does not obviate the relevance of these data to current MDA efforts, because results of a recent trial comparing the anti-filarial effects of diethylcarbamazine alone to diethylcarbamazine plus albendazole showed that 2 years after a single treatment, there was no difference in impact of the two regimens on MF status, with a modestly greater effect of the combination on lowering antigenemia (the proportion of antigen-positive individuals decreased by 10% for diethylcarbamazine alone versus 17% for the combination).[15] When interpreting antibody and antigen data from this and other long-term follow-up studies, it is important to acknowledge that the temporal relationship between drug-mediated killing of adult *W. bancrofti* worms and antigen or antibody clearance from plasma is not known.

MF detected by microscopy of 1-mL blood volumes subjected to filtration decreased to the greatest degree and most persistently of any of the markers studied. Five years after completion of the MDA study, only 4 of 535 individuals were MF positive; 3 of these moved into the study villages after the completion of MDA. Furthermore, no persons who were MF negative before the trial were MF positive 1 year after the fourth annual round of MDA. Similar to MF, there were no antigen- or antibody-negative individuals before MDA who were positive 5 and 10 years after the MDA trial was initiated. In contrast, antigen- and antibody-positive subjects remained positive at the latter time points. In the case of antigen, the lack of change in status likely reflects the slow clearance of the Og4C3 target antigen from the circulation, as reported by others,[16] and the failure to completely eliminate adult worms with five annual rounds of MDA. The latter explanation may be especially relevant to LF in this area of Papua New Guinea, where infection burdens estimated by antigen and MF levels are high relative to other filarial-endemic regions where the assay has been used.[12] It is not yet known why Bm14 antibody persisted in 49% and 31% of individuals observed 1 year after the fourth round and 5 years after the fifth round of MDA (Figure 3). Given that LF is endemic in areas near the villages where the MDA trial was conducted, it is possible the transmission increased after stopping MDA because new residents who have never been treated with anti-filarial medications migrated into the study villages where mosquito-borne transmission was not detected shortly after conclusion of MDA.[17] This is supported by the subsequent discovery of three MF positive post-MDA migrants with MF counts of up to 5,793/mL. It is also possible that transmission increased to pre-MDA levels in those study villages where transmission was highest before commencement of the trial.[18] We cannot, however, differentiate whether the continued presence of antibody is caused by the extended presence of antigen, resumption of transmission, or their combination. We have begun to evaluate entomologic variables that reflect prevailing transmission conditions in these villages using molecular xenomonitoring[19,20] and traditional dissection methods. The observation that the total number of doses of anti-filarial drugs actually consumed does not correlate with conversion from antibody or antigen positive to negative status suggests that individual compliance has little impact on interpretation of these markers. This may not be the case where overall compliance in a population is low. Participation of eligible individuals in the four annual rounds of MDA given from 1994 to 1997 ranged from 77% to 86%.[18]

Few other studies have evaluated parallel changes in the various laboratory tools to monitor human infection status before and after MDA. The largest study to date was conducted in Egypt, which describes changes in MF, Og4C3 antigen, and Bm14 IgG4 antibody after five annual rounds of MDA in four sentinel villages included in the national LF elimination program.[21] Reduction in the MF reservoir was observed such that the currently mooted elimination threshold of 1% prevalence was achieved in one study area (no MF-positive individuals were detected by thick blood smear) and nearly reached in the other (1.2% MF prevalence). With respect to filarial antigen (measured using a card test, not ELISA as in this study), this variable decreased to a greater extent in Egypt than Papua New Guinea. In the highest transmission area of Egypt, pre-MDA and post-MDA antigen-positive rates were 19.0% and 4.8%, whereas in Papua New Guinea, there was no significant change after the fourth round of MDA relative to the pre-MDA prevalence of 86.5%. The higher transmission intensity, greater infection burdens, and lack of albendazole in MDA given in Papua New Guinea may contribute to the apparent lower efficacy of MDA in the latter area. Although additional differences in LF ecology limit the validity of comparing the results of the two studies (e.g., *W. bancrofti* is transmitted by *Culex* mosquitoes in Egypt and by *Anopheles* mosquitoes in Papua New Guinea), it is noteworthy that similar trends in changes of Bm14 antibody suggest that this test is an informative marker to monitor ongoing and recent transmission in sentinel groups of children. In the 7- to 8-year-old children (the youngest group examined) living in the highest transmission site in Egypt, pre- and post-MDA antibody rates fell from 10.0% to 0.4% (MF rates were not described); in Papua New Guinea, where 1- to 6-year-old children were examined, antibody rates fell from 75.0% to 37.0% ($P < 0.01$) and MF rates fell from 26% to 0% ($P < 0.001$). Precise antibody thresholds in the general

population or in sentinel age groups that will be useful in making decisions regarding when MDA may be stopped or should be resumed after cessation of MDA remain to be determined. Bm14 antibody, entomologic outcomes evaluated by molecular xenomonitoring, MDA compliance rates, and ecological variables such as the efficiency of different mosquito vectors to transmit LF should yield quantifiable information that can be incorporated into computational models to predict transmission breakpoints. Such mathematical models will hopefully be sufficiently general that they can be modified to the circumstances that apply to varied ecologies that characterize LF worldwide.[22]

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