

# Prevalence of Bancroftian Filariasis on the Thai-Myanmar Border

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Lymphatic filariasis mainly caused by *Wuchereria bancrofti* and *Brugia malayi* is still a major public health problem in the tropics and subtropics.<sup>1</sup> The disease affects over 120 million people in 73 countries with more than 40 million cases of overt clinical disease.<sup>2</sup> More than 90% of the disease burden is due to *W. bancrofti*. The disease pathology is associated with inflammation and fibrosis of the lymphatic system, resulting in blockage of the lymphatic drain. The majority of infected individuals have asymptomatic microfilaremia which makes the disease difficult to diagnose in the early stages.

Lymphatic filariasis has been acknowledged by the World Health Organization (WHO) as a major public health problem and targeted to be eliminated by the year 2020.<sup>3</sup> Therefore, control programs have been launched worldwide with an emphasis on community-wide treatment with diethylcarbamazine (DEC) and ivermectin or albendazole.<sup>1</sup> To achieve this goal, effective control

**SUMMARY** To achieve the goal of eliminating lymphatic filariasis by the year 2020, close monitoring systems and effective control strategies need to be implemented and the real disease burden needs to be assessed. Bancroftian filariasis is endemic at the Thai-Myanmar border. However, there are only limited data on the prevalence of this disease in Thailand available. We employed microscopic examination, together with ELISA kits to detect *W. bancrofti*-specific Og4C3 circulating antigen and specific anti-filarial IgG4 antibodies to determine the burden of bancroftian filariasis in an endemic area at the Thai-Myanmar border in Umphang District, Tak province, Thailand. A total of 433 Thai-Karen blood samples were analyzed. The microfilarial rate determined by microscope was 6% and the *W. bancrofti*-specific Og4C3 antigenemia rate was 22%, while the specific anti-filarial IgG4 antibody rate was 54%. There were statistically significant higher levels of *W. bancrofti*-specific Og4C3 antigen in the microfilaremic-antigenemic group than in the amicrofilaremic-antigenemic group (unpaired Student's t-test;  $p < 0.001$ ), similar to the specific anti-filarial IgG4 antibody results (unpaired Student's t-test;  $p < 0.001$ ). A statistically significant correlation of moderate degree between the presence of *W. bancrofti*-specific Og4C3 antigen and of specific anti-filarial IgG4 antibody was found in the amicrofilaremic group ( $r = 0.474$ ,  $p < 0.001$ ), but not in the microfilaremic group ( $r = 0.291$ ,  $p > 0.05$ ). Our study revealed a very high prevalence of bancroftian filariasis in this endemic area and thus emphasized the importance of using highly sensitive and specific diagnostic tools to evaluate the true prevalence of the disease.

strategies and close monitoring systems need to be implemented and the real disease burden needs to be assessed. Close monitoring of the intervention programs requires sensitive and cost-effective diagnostic assays. The conventional method for diagnosis of lymphatic filariasis is by detecting microfilariae in nocturnal peripheral blood.<sup>4-6</sup> The reason

for this is that, in most geographical areas, *W. bancrofti* microfilariae have a nocturnal periodicity or subperiodicity, with the highest concentra-

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tion in the peripheral blood at night, and few or no microfilariae during the day.<sup>7</sup> Besides being time-consuming and tedious, the conventional parasitological procedures fail to identify amicrofilaremic infections or individuals with very low microfilaria levels.<sup>8</sup>

Detection of the circulating filarial antigen has now emerged as an alternative method for the diagnosis of *W. bancrofti* infection.<sup>6,9</sup> Like the conventional method, the antigen assay is a good indicator for active infection. Commercial kits utilizing specific monoclonal antibodies for *W. bancrofti* antigen detection are now available.<sup>10,11</sup> An enzyme-linked immunosorbent assay (ELISA) based on Og4C3 monoclonal antibody<sup>11</sup> has been used to diagnose microfilaremic as well as amicrofilaremic individuals with high specificity and sensitivity.<sup>4,11-13</sup> The sensitivity of this antigen assay is the same whether serum samples are collected during the day or at night.<sup>14</sup> Besides the Og4C3 ELISA, another antigen assay for the diagnosis of *W. bancrofti* has been developed as immunochromatographic (ICT) Filariasis card.<sup>10</sup> The efficacy of the ICT Filariasis card is comparable to the *W. bancrofti*-specific Og4C3 antigen test.<sup>10</sup> Due to the ease of use, this card test is a promising point-of-care (PoC) diagnostic tool highly suitable for remote areas.<sup>10,15-17</sup> Moreover, it provides results in 15 minutes using finger-prick blood. However, the *W. bancrofti*-specific Og4C3 ELISA shows a slightly higher sensitivity<sup>16,18</sup> especially in detecting amicrofilaremic,<sup>18</sup> compared to the ICT Filariasis card test. For multiple sample testing, such as during and after mass treatment programs, the *W.*

*bancrofti*-specific Og4C3 ELISA is more practical. The Og4C3 ELISA seems to be highly valuable for large scale screening<sup>17</sup> as well. Therefore, it depends on the situation which of these two antigen tests is more appropriate to use.

Serological assays based on the detection of anti-filarial IgG antibodies are widely used for epidemiological and diagnostic purposes. The main advantage of the anti-filarial IgG antibody detection is the ability to detect filarial infection during the incubation period and in occult filariasis (tropical pulmonary eosinophilia [TPE]), in which microfilariae are not found in the peripheral blood. Furthermore, due to the long persistence of antibodies, past as well as current cases can be detected by this ELISA. However, the anti-filarial antibody test is known to have a relatively low specificity. Specific subclasses of IgG antibodies have been shown to improve the specificity over total IgG antibodies for the diagnosis of many parasitic infections, such as ascariasis,<sup>19</sup> echinococcosis,<sup>20</sup> leishmaniasis,<sup>21</sup> and gnathostomiasis.<sup>22</sup> The specific anti-filarial IgG4 antibody have also been shown to enhance the sensitivity of the immunodiagnostic assay for lymphatic filariasis.<sup>5,6,23-28</sup> However, the specific anti-filarial IgG4 antibody assay has a lower specificity than the parasitological or antigenic diagnostic techniques.<sup>29</sup> The serum levels of the specific anti-filarial IgG4 antibodies are elevated in persons with active *W. bancrofti* infection<sup>22-24</sup> and decline following treatment with DEC.<sup>30</sup>

In Thailand, lymphatic filariasis has been well controlled, re-

sulting in a low prevalence rate of 1.62 per 100,000 population,<sup>31</sup> as assessed by the conventional microscopic method. The data indicate that only 86,000 individuals in 106 sub-districts are at risk of acquiring lymphatic filariasis. Areas of active transmission of bancroftian filariasis are limited to certain districts in Tak, Kanchanaburi, and Mae Hong Son provinces, located in the North-west of Thailand at the Thai-Myanmar border. Individuals in endemic areas receive bi-annual DEC treatment and health education including prevention and control of lymphatic filariasis from public health personnel. Among the active transmission areas of bancroftian filariasis in Thailand, Umphang district in Tak province, is considered to be one of the most afflicted. The main reasons are its distant location, difficult access and that it is covered with rain forests. Records from the Thai Meteorological Department (TMD) during 1998 to 2000 indicate that Umphang district had higher annual rainfalls than Kanchanaburi and Mae Hong Son provinces.<sup>31</sup> Umphang district has about 1,399.5 mm rainfall with a total of 164 rainy days per year. Such conditions create many water reservoirs serving as breeding sites for *Aedes niveus*, the main mosquito vector for bancroftian filariasis in Thailand.<sup>32</sup> However, very few studies on bancroftian filariasis in this remote areas have been done,<sup>33</sup> and therefore the epidemiology of the disease is poorly known. The real situation of lymphatic filariasis in endemic areas in Thailand has not been investigated using highly sensitive and specific tests like the *W. bancrofti*-specific Og4C3 circulating antigen and the specific anti-filarial IgG4 antibody assays.



To accurately assess the real burden of the bancroftian filariasis situation, we studied the prevalence of bancroftian filariasis in a Thai-Karen population residing in sub-districts of Umphang district, Tak province, Thailand, using the conventional microscopic method, the *W. bancrofti*-specific Og4C3 ELISA, and the ELISA for anti-filarial IgG4 antibodies, and discuss their results in relationship to each other.

## MATERIALS AND METHODS

### Study area and population

Two villages of Mae Chan sub-district, Umphang district, Tak province at the Thai-Myanmar border, Thailand (Fig. 1), were selected for the study. The Umphang district is located about 165 km from Mae Sot district of Tak province. However, it takes about 4-6 hours to get there from Mae Sot by a tortuous secondary road. The Center of Vector Borne Disease Control (VDC) 18 is located in Mae Sot district, about 500 km northwest of Bangkok. The villagers were very cooperative and pleased with our visit.

Among the study population, 55% (238/433) were men and 45% (195/433) were women. The majority of the population was between 15 and 30 years (44%). Nineteen percent were children less than 15 years old. Most of individuals living here worked on agriculture.

Verbal informed consent was obtained from each individual or child's parents or guardian in the presence of two witnesses. All participants were informed about the purpose of this study and the danger

of lymphatic filariasis. Individuals who were microfilaremic were treated with the standard treatment (DEC). This study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok. We performed the study in cooperation with Filariasis Division, and VDC 18 (Mae Sot district), Department of Communicable Disease Control (CDC), Ministry of Public Health, Thailand.

### Sample collection

A total of 539 individuals were recruited for the study, but only 433 allowed us to obtain blood samples. The sera of the blood samples were separated and stored at -20°C until use. Negative control sera were obtained from non-infected healthy people living in non-endemic areas. Sera from patients infected with other parasites, diagnosed by stool examination<sup>35-36</sup> and Giemsa stain of thin and thick blood films,<sup>5,6,27,28,34</sup> including *B. malayi*, *Onchocerca volvulus*, *Opisthorchis viverrini*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Giardia lamblia* and hookworms, were also tested.

### Detection of microfilariae

Blood samples were collected between 8.00-12.00 p.m., the preferential time for the detection of the nocturnal subperiodic microfilariae. Two to five milliliters of venous blood were obtained from each individual by a sterile technique. For identification of microfilariae, thick blood films were prepared in duplicate as described previously.<sup>5,6,27,28,34</sup> Briefly, about 60 microliters of each of the 433 blood samples were smeared onto micro-

scope slides in duplicate. After being air-dried, the slides were stored at room temperature until examination in the laboratory. The microscope slides were stained with Giemsa's technique. Species identification was performed independently by 2 individuals.

We had difficulties to be able to process all 433 blood samples for all 3 tests, because of transportation problems and the limited amounts of blood obtained. All 433 blood samples were examined microscopically for the microfilariae, 265 blood samples were also tested for *W. bancrofti*-specific Og4C3 antigen and 178 samples were tested by all 3 methods.

### Detection of *W. bancrofti*-specific Og4C3 antigen

The *W. bancrofti*-specific Og4C3 antigen was detected and measured by using a specific Og4C3 monoclonal antibody-based sandwich ELISA according to the manufacturer's instructions (Trop-Bio Pty Ltd, Townsville, Queensland, Australia). The results were expressed in arbitrary antigen units per milliliter. The antigen units were calculated from a standard curve generated by using known concentrations of *Onchocerca gibsoni* antigen as standard. Positive samples had *W. bancrofti*-specific Og4C3 antigen titers over 100 U/ml. All samples were performed in duplicate.

### Detection of specific anti-filarial IgG4 antibodies

Detection and quantification of the specific anti-filarial IgG4 anti-



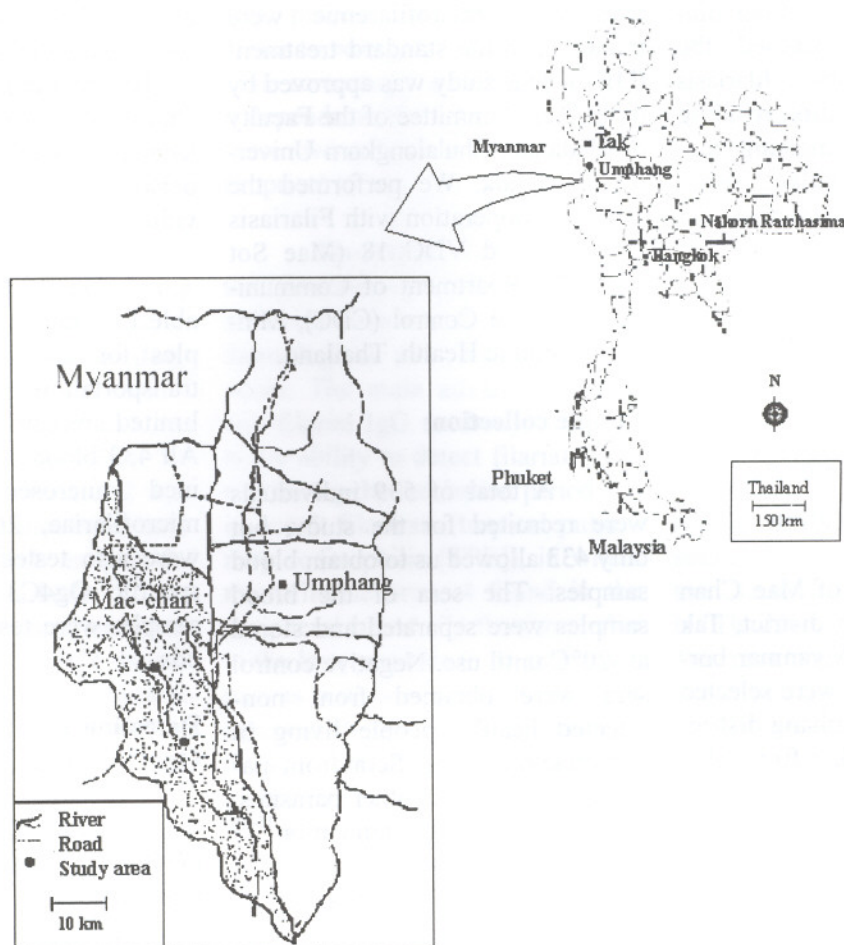


Fig. 1 Location of the study areas at Mae Chan sub-district, Umphang district, Tak province, Thailand.

bodies were performed by ELISA as described previously<sup>5</sup> with some modifications. Briefly, 100 microliters of adult *B. malayi* extract in carbonate buffer (1 µg/ml) were coated onto each well of microtiter plates and incubated at 4°C overnight. One hundred microliters of each serum sample were added to each well at a 1:100 dilution. Anti-human IgG4-horseradish peroxidases (HRP) conjugate (Zymed, South San Francisco, California, USA)(1:2,000) was used as secondary

antibody. A substrate mixture (O-phenylenediamine; OPD) was added to each well and left at room temperature for 30 minutes. The reaction was stopped by adding 50 microliters of 4 N H<sub>2</sub>SO<sub>4</sub>. The optical density (O.D.) was read at the wavelength of 490 nm. The O.D. for positive cases was set above the mean + 3 S.D. of the control sera from healthy volunteers. Specific anti-filarial IgG4 antibody results were expressed in arbitrary units per milliliter. The anti-filarial IgG4 antibody units were cal-

culated from a standard curve generated by using known concentrations of IgG4 antibodies as standard. All samples were tested in duplicate.

#### Data analysis

Data were recorded and analyzed using Microsoft Excel 6.0 program and SPSS for Windows. Unpaired t-test was used to compare log-transformed Og4C3 antigen and anti-filarial IgG4 antibody

levels between amicrofilareemics and microfilareemics with a  $p < 0.05$  taken as statistically significant. The correlation between the specific anti-filarial IgG4 antibody units and the *W. bancrofti*-specific Og4C3 antigen units was determined by Pearson's correlation test. Furthermore, Cohen's kappa coefficient was computed to assess the agreement between the positive/negative results from the *W. bancrofti*-specific Og4C3 antigen assay and that from the specific anti-filarial IgG4 antibody assay.

## RESULTS

### Prevalence of bancroftian filariasis assessed by microfilarial rate

Out of 433 individuals, 24 (6%) individuals were positive for the microscopic examination of microfilariae (Table 1). The majority of positive individuals were 30-45 years old (9%). The microfilaremia was more common in men than in women (1.67: 1) (data not shown). All positive specimens for microfilariae were *W. bancrofti*. All of the non-infected healthy people and patients with other parasites were negative for both the *W. bancrofti*-specific Og4C3 antigen and anti-filarial IgG4 antibodies.

### Prevalence of bancroftian filariasis assessed by the *W. bancrofti*-specific Og4C3 ELISA

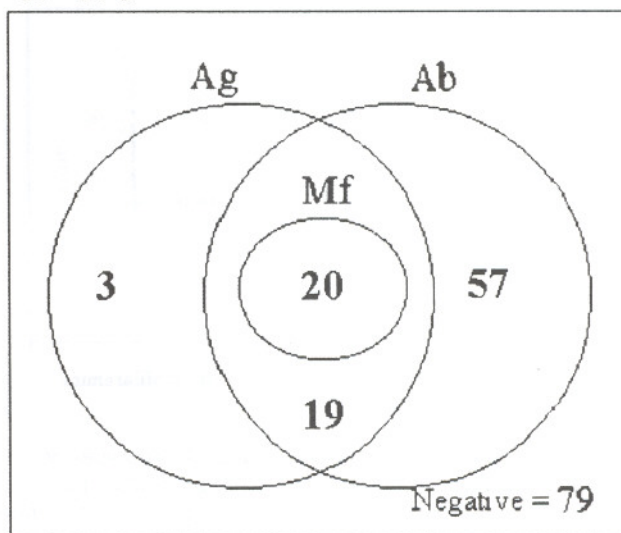
The ELISA for the *W. bancrofti*-specific circulating antigen (Og4C3) was performed in 265 blood samples. Antigenemia was detected in 58 (22%) patients (Table 1), which was three-fold higher than the conventional microscopic method. All of the microfilaremic

**Table 1** Prevalence of bancroftian filariasis in the study areas

Test	No. examined	No. positive (%)
Microscopic examination for microfilariae	433	24 (6)
<i>W. bancrofti</i> Og4C3 ELISA	265	58 (22)
ELISA for anti-filarial IgG4 antibodies	178	96 (54)

Note: See Materials and Methods for details of study population and tests.

N=178



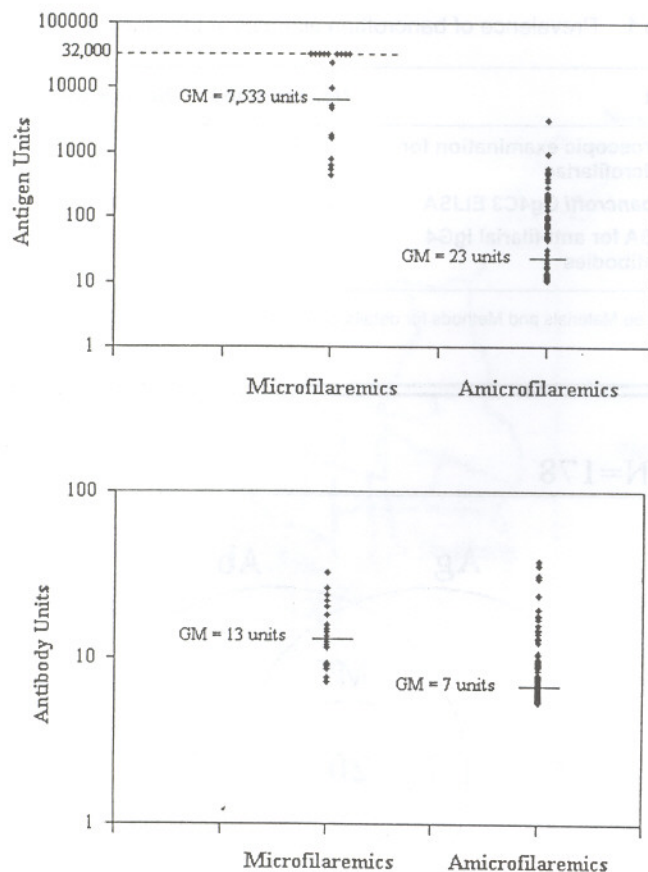
**Fig. 2** Venn Diagram showing the numbers of Mf (Microfilariae), Ag (Og4C3 antigen), and Ab (specific anti-filarial IgG4 antibodies). N = number of samples (178) that had enough blood for testing all 3 diagnostic tests. Numbers in circles indicate numbers of positive cases.

individuals were positive for the *W. bancrofti*-specific Og4C3 antigen (Fig. 2). The *W. bancrofti*-specific Og4C3 antigen was also detected in 15% (34/232) of amicrofilaremic individuals. The age-adjusted prevalence of the *W. bancrofti*-specific Og4C3 antigen was highest (35%) in the 45-60 years age group (data not shown).

### Prevalence of bancroftian filariasis assessed by ELISA for specific anti-filarial IgG4 antibodies

The ELISA for specific anti-filarial IgG4 antibodies was used to assess the immune response to bancroftian filariasis in 178 individuals. Ninety-six (54%) individuals were positive for the specific anti-filarial





**Fig. 3** Scattergram of Og4C3 antigen levels (A) and specific anti-filarial IgG4 antibody levels (B) classified by their microfilaremic status. Horizontal bars are geometric mean (GM) values. Og4C3 antigen levels and anti-filarial IgG4 antibody levels in the microfilaremic group were significantly higher than in the amicrofilaremic group ( $p < 0.001$ ).

IgG4 antibodies (Table 1). Among this group, 20 individuals were microfilaremic-antigenic, 19 individuals were amicrofilaremic-antigenic, and 57 individuals were amicrofilaremic non-antigenic (Fig. 2).

#### Levels of *W. bancrofti*-specific Og4C3 antigen and anti-filarial IgG4 antibodies in microfilaremic patients

To study the association between the microfilaremic status and the *W. bancrofti*-specific Og4C3

antigen levels, we compared the geometric means of the *W. bancrofti*-specific Og4C3 antigen levels in microfilaremic and amicrofilaremic groups. The range of the *W. bancrofti*-specific Og4C3 antigen levels was 439 to  $> 32,000$  units in the microfilaremic group, while it was 10-3,156 units in the amicrofilaremic group (Fig. 3). The geometric mean of the *W. bancrofti*-specific Og4C3 antigen levels among the microfilaremic group (7,533 units) was significantly higher than that in

the amicrofilaremic group (23 units) (unpaired Student's *t*-test;  $p < 0.001$ ) (Fig. 3). Similar to the *W. bancrofti*-specific Og4C3 antigen, the geometric mean of the specific anti-filarial IgG4 antibodies was also significantly higher in the microfilaremic group than in the amicrofilaremic group ( $p < 0.001$ ) (Fig. 3). When comparing between men and women, there was no statistically significant difference of both the *W. bancrofti*-specific Og4C3 antigen and the specific anti-filarial IgG4 antibody levels (data not shown).

#### Relationship between the *W. bancrofti*-specific Og4C3 antigen and the specific anti-filarial IgG4 antibody levels

All of the microfilaremic cases were positive for both the *W. bancrofti*-specific Og4C3 antigen and the specific anti-filarial IgG4 antibodies (Fig. 2). The specific anti-filarial IgG4 antibodies were also detected in 48% (76/158) of amicrofilaremic individuals. In this group, 25% (19/76) were antigenemic individuals and 75% (57/76) were non-antigenemic individuals. Some (1.8%; 3/158) of the amicrofilaremic individuals were negative for the specific anti-filarial IgG4 antibodies but positive for the *W. bancrofti*-specific Og4C3 antigen. Similar to the antigenemic data, the highest age-adjusted positive rate (87%) for the specific anti-filarial IgG4 antibodies was in the 45-60 years age group (data not shown). Furthermore, the *W. bancrofti*-specific Og4C3 antigenemia pattern and the specific anti-filarial IgG4 immune response increased with age.

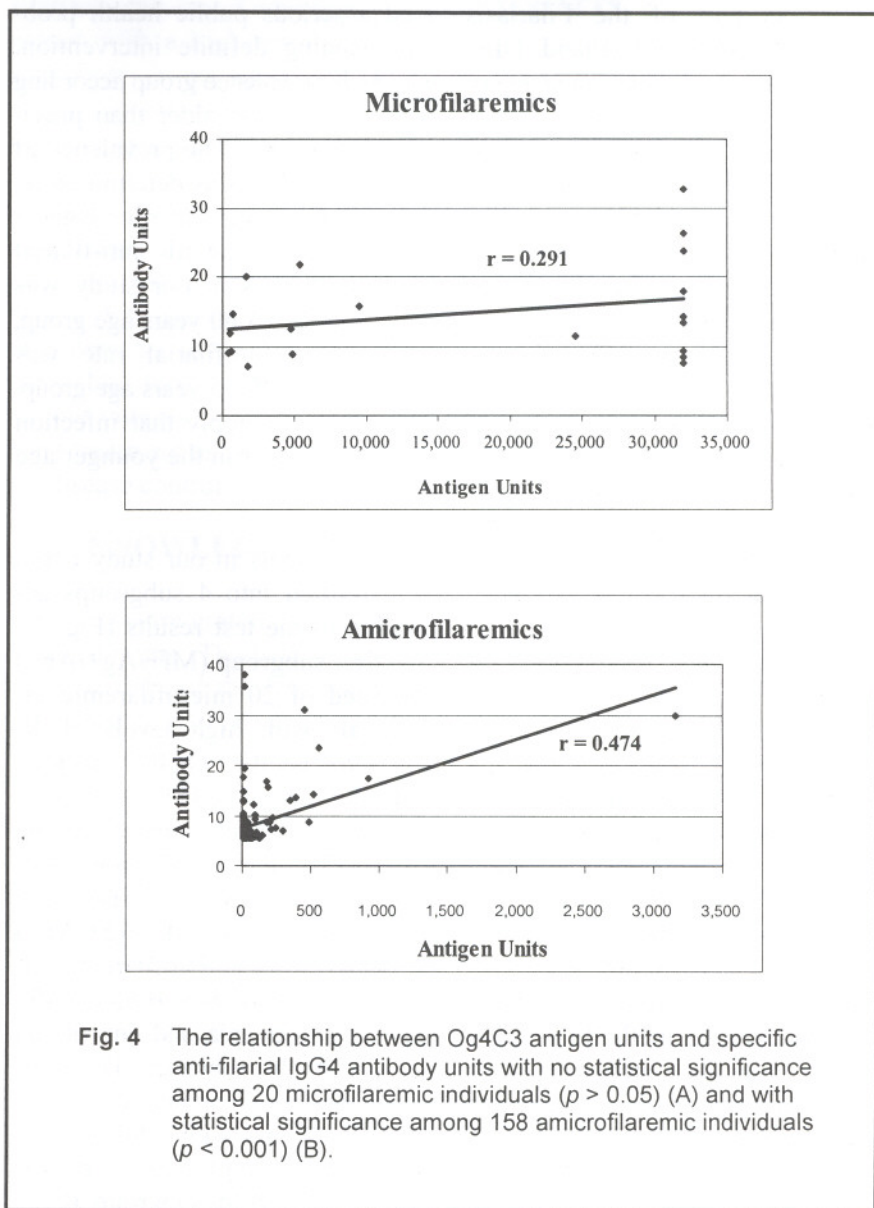
A statistically significant

correlation of moderate degree was found between the *W. bancrofti*-specific Og4C3 antigen units and the specific anti-filarial IgG4 antibody units in the amicrofilaremic group ( $r = 0.474$ ,  $p < 0.001$ ), while that in the microfilaremic group showed no statistically significant correlation ( $r = 0.291$ ,  $p > 0.05$ ) (Fig. 4).

We further analyzed the agreement of positive/negative results from both the *W. bancrofti*-specific Og4C3 antigen and the specific anti-filarial IgG4 ELISAs by using Cohen's kappa statistics. For the amicrofilaremic group, the results showed that the level of agreement between the results from the *W. bancrofti*-specific Og4C3 antigen assay and the specific anti-filarial IgG4 antibody assay had a fair statistical significance (kappa = 0.219,  $p < 0.001$ ) (data not shown). Among the microfilaremic group, all individuals were positive for both the *W. bancrofti*-specific Og4C3 antigen and the specific anti-filarial IgG4 antibodies.

## DISCUSSION

The conventional parasitological method possibly fails to define the real impact of the disease. This is an important impediment of disease control strategies. The prevalence rate of Thai-Karens living in Umphang district was significantly higher when assessed by advanced techniques. Our results agreed with previous reports that a higher prevalence of the disease could be demonstrated by the antigen tests compared to the microscopic examination of microfilariae.<sup>5,6,10,12,14,37-39</sup> Therefore, advanced diagnostic techniques are useful for the assessment of the true prevalence of the



disease.

Diagnosis of lymphatic filariasis by using the circulating antigens as markers provides several advantages. They can be used to determine the infectious status of individuals with a low or no microfilaremia.<sup>14,40</sup> Furthermore, blood specimens can be collected during daytime.<sup>14,37</sup> Thus a large numbers of specimens can be obtained in a short period of time. As part of the global elimination program, the an-

tigen testing is therefore a useful rapid screening tool for defining the real prevalence and distribution of *W. bancrofti*.<sup>10,41</sup> In this study, we used the Og4C3 ELISA which seemed practical for large scale screening. Serological assays based on the detection of anti-filarial IgG antibodies are widely used for epidemiological and diagnostic purposes. Therefore, we also used an ELISA for anti-filarial IgG4 antibodies to study the epidemiology of the disease in this endemic area.



As part of the Filariasis Control Program in Thailand, DEC mass treatment is performed every 6 months in endemic areas, while prevalence assessment is performed randomly in some villages. The study area selected is determined by the prevalence of the disease during the past years. Sometimes the study area is expanded to cover more areas. However, the data collected over several years is used to determine the overall situation of the disease in each endemic area. The prevalence of bancroftian filariasis in this endemic area during the years 1992-1999 was 0.42-2.97%<sup>31</sup> using the conventional microscopic method.

Very few studies on the prevalence of bancroftian filariasis in endemic areas of Thailand have been reported.<sup>33</sup> We found that the prevalence of bancroftian filariasis in Mae Chan sub-district was 6% by the microscopic method, compared to 22% by the *W. bancrofti*-specific Og4C3 antigen test, and to 54% by the anti-filarial IgG4 antibody assay. A previous study, using the microscopic method in comparison to another antigen test (the ICT Filariasis card test) showed a similar pattern of disease burden.<sup>33</sup> Therefore, it is evident that the microscopic method underestimated the prevalence of filariasis in this population more than three-fold. Our data also indicated that one-fifth of the study population had active *W. bancrofti* infection as assessed by the antigen assay. Furthermore, the 54% prevalence assessed by the specific IgG4 antibody rate signified that more than half of the population were either actively infected or had had a past infection of bancroftian filariasis. The high burden of the disease in this endemic area should be consid-

ered a serious public health problem needing definite intervention. The high-prevalence group according to our study was older than previously reported.<sup>31</sup> The prevalence of bancroftian filariasis determined by the *W. bancrofti*-specific Og4C3 antigen and the specific anti-filarial IgG4 antibodies in our study was highest in the 45-60 years age group, while the microfilarial rate was highest in the 30-45 years age group. This seems to imply that infection rates were lower in the younger age groups.

Patients in our study could be classified into 4 subgroups according to the test results (Fig. 2). The first subgroup (Mf+/Ag+/Ab+) consisted of 20 microfilaremic individuals with high levels of *W. bancrofti*-specific Og4C3 antigen and anti-filarial IgG4 antibodies. Obviously, this subgroup represents active *W. bancrofti* infections with high levels of microfilariae. The second subgroup (Mf-/Ag+/Ab+) consisted of 19 amicrofilaremic individuals with *W. bancrofti*-specific Og4C3 antigenemia and anti-filarial IgG4 antibody responses. This subgroup may represent active *W. bancrofti* infections with adult worms but producing too few or no microfilariae. The third subgroup (Mf-/Ag-/Ab+) consisted of 57 amicrofilaremic individuals with variable levels of anti-filarial IgG4 antibodies, without detectable Og4C3 antigen levels. This subgroup possibly represents past-infected individuals who had already eliminated the *W. bancrofti*-specific Og4C3 antigen from their blood, but remained anti-filarial IgG4 antibody positive. Alternatively, this group may have had too low levels of *W. bancrofti* parasites to be detected by either the microscopic or the antigen meth-

ods. The last subgroup (Mf-/Ag+/Ab-) had *W. bancrofti*-specific Og4C3 antigen in the blood circulation, but had no detectable microfilaremia, nor a detectable anti-filarial IgG4 antibody response. It is possible that this group was in the early period, or window period of the infection, or had too low levels of antibody response to be detected.<sup>25,41</sup> Further studies are needed to clarify the issues raised.

From Fig. 4, a statistically significant correlation of moderate degree was found between the *W. bancrofti*-specific Og4C3 antigen units and the anti-filarial IgG4 antibody units of the amicrofilaremic group ( $r = 0.474$ ,  $p < 0.001$ ). However, there was no statistically significant correlation in the microfilaremic group ( $r = 0.291$ ,  $p > 0.05$ ). Some microfilaremic individuals had very high levels of *W. bancrofti*-specific Og4C3 antigen with low levels of specific anti-filarial IgG4 antibodies (Fig. 4). These results were similar to previous reports.<sup>25,42</sup> It has been proposed that this discrepancy is either caused by an association of the increased antigen load in microfilaremic individuals with a down-regulation of the antibody production,<sup>42</sup> or simply by the early infectious stage of the microfilaremic individuals tested. However, when the agreement between the *W. bancrofti*-specific Og4C3 antigen and the specific anti-filarial IgG4 antibody results was analyzed in terms of number of positive/negative cases by using Cohen's kappa statistic, the results showed that the level of agreement in amicrofilaremic individuals only had a fair statistical significance (kappa = 0.219,  $p < 0.001$ ), while all of the microfilaremic individuals were positive for both the *W. bancrofti*-specific



Og4C3 antigen and the specific anti-filarial IgG4 antibodies (100% agreement). This data suggested that the *W. bancrofti*-specific Og4C3 antigen and the specific anti-filarial IgG4 antibodies were correlated with microfilaremic status. When the data were analyzed in terms of magnitude of *W. bancrofti*-specific Og4C3 antigen levels and specific anti-filarial IgG4 antibody levels, microfilaremic patients had significantly higher *W. bancrofti*-specific Og4C3 antigen levels than amicrofilaremic patients ( $p < 0.001$ ) (Fig. 3). This could be explained by that the parasite burden in microfilaremic patients was higher, therefore, more antigens were released into the blood circulation. Similar to the *W. bancrofti*-specific Og4C3 antigen units, the microfilaremic patients had higher levels of specific anti-filarial IgG4 antibodies than amicrofilaremic patients with statistical significance ( $p < 0.001$ ) (Fig. 3). Moreover, we found that the specific anti-filarial IgG4 antibody levels increased with the *W. bancrofti*-specific Og4C3 antigen levels in both amicrofilaremic and microfilaremic individuals. Our results showed that both the *W. bancrofti*-specific Og4C3 antigenemia and the specific anti-filarial IgG4 antibodies correlated with the microfilaremic status. Furthermore, our data supported that both the *W. bancrofti*-specific Og4C3 antigen levels, and the specific anti-filarial IgG4 antibody levels correlated with active infection.<sup>25</sup>

Our data showed a high prevalence of bancroftian filariasis in the endemic area investigated emphasizing the importance of close monitoring of the disease in this area. To monitor and evaluate the elimination program of lymphatic

filariasis, the use of advanced techniques with a high sensitivity and specificity is necessary. Furthermore, the test method used should be practical for a field study setting in that a lot of samples can be easily tested and blood samples taken at any time of the day. Finally, it is also necessary to improve the access for people to health care providers, as well as the access for the public health personnel to the remote villages in order to provide mass treatment as well as education for disease control.

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