A comparison of the Og4C3 antigen capture ELISA, the Knott test, an IgG4 assay and clinical signs, in the diagnosis of Bancroftian filariasis

P. Turner[1], *B. Copeman*[2], *D. Gerisi*[3], *R. Speare*[1]

[1] Anton Breinl Centre for Tropical Health and Medicine, James Cook University of North Queensland, Townsville, Australia; [2] Graduate School of Tropical Veterinary Science and Agriculture, James Cook University of North Queensland, Townsville, Australia; [3] Rumginae Health Centre, Kiunga, Western Province, Papua New Guinea

Abstract

To compare methods for diagnosing Bancroftian filariasis, six hundred and seventy four people from rural areas of Papua New Guinea were screened using microfilaraemia, the Og4C3 antigen capture ELISA, an ELISA to detect IgG4 specific antibodies and clinical examination. Both ELISA tests detected around twice the number of positive cases than those detected by the presence of microfilariae alone. No correlation was found with clinical signs and laboratory tests. This study raises concern over the underestimation of the prevalence of filariasis due to ineffective diagnostic criteria. The two ELISA tests should be of value in epidemiological surveys and for monitoring filariasis control programs.

Introduction

The standard technique used to diagnose lymphatic filariasis is the detection of microfilariae in the peripheral blood of an infected individual. In most regions a significant problem with this technique is nocturnal periodicity. Subjects bled outside the times of 22.00 to 02.00 show a marked reduction in the density of microfilariae (Mf) in the blood with a large proportion of true positives being deemed negative (Manson, 1880). A further difficulty is the inability to detect prepatent or occult infections. Bancroftian filariasis is therefore often under reported using microfilaraemia as a diagnostic test.

Wuchereria bancrofti infections have been reported in many areas of Papua New Guinea (Forsyth 1987; Kazura et al., 1984; Taufa, pers. comm. 1991). In the Western Province of Papua New Guinea Bancroftian filariasis is hyperendemic (Schurrkamp et al., 1987), with prevalence as indicated by the presence of microfilariae averaging about 30 % (Schurrkamp et al., 1987; Knight et al., 1979; Turner, 1991).

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This study examines 4 methods of diagnosing Bancroftian filariasis; clinical examination, the detection of microfilaraemia, the Og4C3 antigen capture ELISA which detects adult worm antigens from *W. bancrofti* (More and Copeman, 1990) and an antibody capture ELISA detecting IgG4 filarial specific antibody. The detection of mf in the peripheral blood is the recognised standard test and the two ELISA's were assessed against it. The ELISA tests were chosen because one detected antigen and the other detected antibody against filarial worms. The detection of filarial specific IgG4 antibodies is an indicator of active infection (Kwan-Lim et al., 1990). The detection of IgG4 antibodies has also been shown to reduce cross reactivity with non-filarial parasite antibodies (Lal and Ottesen, 1988) due to the high concentration of filarial specific IgG4 antibodies found in filarial infections.

The results from this study indicate that microfilaraemia, the standard test for the diagnosis of a filarial infection underestimates the prevalence of infection with *W. bancrofti* by almost 50 %. The restuls also suggest that ELISA tests on serum are of value in the detection of prepatent and occult infections.

Materials and methods

Subjects

Three hundred and twenty males and 354 females with an age range of 5 to 64 years from three rural villages, Rumginae, Senamrae and Dome, in the Western Province, Papua New Guinea were chosen for inclusion in the survey. Children less than 5 years were excluded. Procedures were explained to all participants and the testing was voluntary. All individuals volunteering for the testing were included in the survey. The age distribution found in this survey group was representative of the general population found in Papua New Guinea (Papua New Guinea Health Plan, 1991).

Each individual was examined clinically for lymphadenopathy, hepatosplenomegaly and limb enlargement while genital changes were assessed in men only. Demographic details including age and sex were also obtained. A plastic hospital identity bracelet, marked with a unique number, was used to identify individuals when they returned after 10.00 pm for their nocturnal blood collection. Blood was collected from an anticubital vein and placed into EDTA tubes for the detection of microfilaraemia and plain tubes for serology. Serum collected was frozen at – 20 °C until it could be stored at – 70 °C in the laboratory.

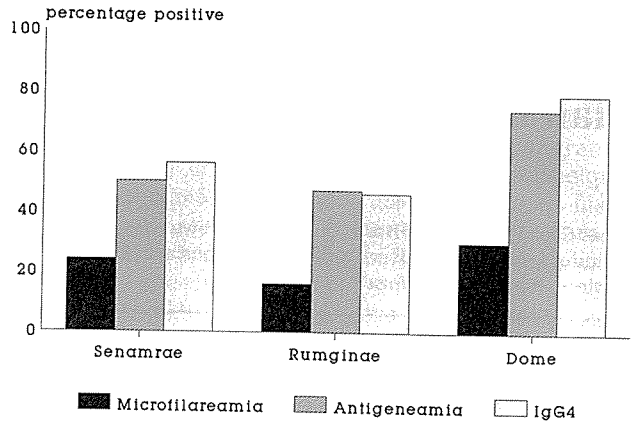

Fig. 1 Prevalence of microfilaraemia, antigenaemia and IgG4 positivity at Senamrae, Rumginae and Dome, three rural villages in the Western Province of Papua New Guinea.

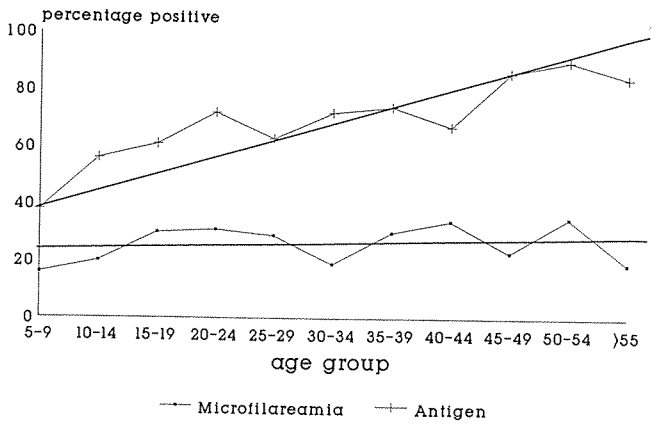

Fig. 2 The prevalence and trends of microfilaraemia antigenaemia and IgG4 positivity by age in 674 Papua New Guineans from three rural villages in the Western Province of Papua New Guinea.

Laboratory analysis

Og4C3 antigen capture ELISA The Og4C3 antigen capture ELISA has been described by More and Copeman (1990). Forty sera from Goroka, an area of Papua New Guinea where filariasis is not endemic, were tested to rule out cross reactivity with possible common antigens found in Papua New Guineans. All of the sera were found to be negative.

Detection of microfilariae A quantitative count of microfilariae per ml of blood was obtained using the modified Knott technique for 1 ml of blood (Mansons-Bahr and Bell, 1987).

IgG4 assay for filariasis Antigen preparation: Live adult heartworm, *Dirofilaria immitis*, were collected from the heart of a sacrificed dog naturally infected in the Townsville region. The worms were washed in ice cold phosphate buffered saline (PBS) at pH 7.2 and were subsequently crushed into a pulp using a mortar and pestle. The pulp was suspended in ice cold PBS and stored at −20 °C. The thawed pulp suspension was sonicated for 5 minutes to bring the somatic antigens into solution. The suspension was centrifuged at 14,000 rpm for 10 minutes,the supernatant fluid frozen at −70 °C until required for ELISA plate coating and the pellet discarded.

ELISA technique Protein estimation of the supernatant fluid was undertaken using the Pierce protein estimation kit. After estimation the solution was diluted to 1 µg per ml using PBS at pH 7.2. The diluted solution was used to coat around bottom 96 well polystyrene ELISA plates (Nunc). One hundred µl of coating solution was placed in each well and incubated overnight at 4 °C. The coated ELISA plates were washed four times with PBS tween 20 (Ajax Chemicals) using the Behring BEP2 automated Elisa Processor. The plates were blocked with 100 µl of 5 % casein w/v with PBS for 30 minutes at 37 °C. The plates were washed and 100 µl of test serum and controls, diluted 1:100 with PBS, pipetted into duplicate wells. After a 2 hour incubation at room temperature the plates were washed and 100 µl of a horse radish peroxidase (HRPO) conjugated anti human IgG4 monoclonal antibody diluted to 1:1000 v/v with PBS tween 20 was placed into each well. This was incubated at 37 °C degrees for 60 minutes and a further washing with PBS tween 20 undertaken. One hundred microlitres of TMB substrate solution (0.4 mM 3.3'. 5.5'-tetramethylbenzadine dihydrochloride and 0.004 % (v/v) H2O2 in 0.1 M acetate buffer, pH 5.6) was added to each well and incubated for 30 minutes before the reaction was stopped with 50 µl of 2 M H_2SO4. The absorbende was analysed using the Behring BEP2 ELISA plate reader at 450 nm. A positive cut-off was assigned as mean pl 3 standard deviations of the negative control.

Clinical examination Individuals in the surv were examined to assess any chronic pathology associated wi Bancroftian filariasis. Any signs of limb enlargement or geni enlargement were noted. The spleen was palpated and grad using Hackett's score. The liver was also palpated and the lev of hepatomegaly graded in cm below the rib cage. The maj subcutaneous lymph nodes, including parotid, cervical, menta submaxillary, axillary, epitrochlear, inguinal and popliteal we palpated and classified as normal or enlarged using the followi criteria: mental and epitrochlear > 0.5 cm, inguinal > 2 cm an the remainder > 1 cm.

Analysis of results All data were scored an analysed using the Paradox relational database and the Parast statistical package. All statistical analysis including Chi squar and the t test was at the 95 % confidence interval.

Results

The prevalences of microfilaraemia, positive Og4C3 antigen and IgG4 ELISA's are illustrated i Fig. 1. The prevalences of filarial infection was found t be significantly higher at Dome (Student's t test $P < 0.05$) in all three tests when compared to Rumginae but was no found to be different from Senamrae. The mean preva lence of microfilaraemia for all participants was found t be 26 % with a mean filarial density of 546 per ml ± 119 of blood (n = 175). The overall prevalence of antigenaemi in three communities was 57 % ± 15 % (n = 384) with th prevalence of IgG4 positivity found to be 60 % ± 15 % (n = 404). There was no significant difference (Chi square) between prevalences as determined by antigenaemia an by IgG4 positivity. No relationship was found between th density of microfilariae and the absorbance of the Og4C3 antigen ELISA or the IgG4 assay. There were approximately twice the number of positives as indicated by th two ELISA's when compared to positives by the Knott test. The Og4C3 ELISA detected all the cases of microfilaraemia in the survey while the IgG4 ELISA failed t detect 5 % (n = 33) of those showing positive microfilaraemia.

Table 1 The prevalence of various clinical signs in 674 Papua New Guineans in the western province of Papua New Guinea.

Clinical signs		Positive	Prevalence
Limb enlargement		3	0.0004 %
Genital changes		2	0.003 %
Hepatomegaly		98	14 %
Splenomegaly		314	47 %
Lymph node enlargement			
Axillary	(> 1 cm)	344	51 %
Cervical	(> 1 cm)	131	19 %
Epitrochlear	(> 0.5 cm)	165	24 %
Inguinal	(> 2 cm)	468	70 %
Mental	(> 0.5 cm)	67	10 %
Parotid	(> 1 cm)	62	9 %
Popliteal	(> 1 cm)	142	21 %
Submaxillary	(> 1 cm)	149	22 %

The results from the clinical examinations are illustrated in Table **1**. There was no significant relationship found between positive laboratory results and clinical signs.

The prevalence of antigenaemia and IgG4 positivity increased with age while the prevalence of microfilaraemia remained constant over all ages (Fig. **2**). There was no cross reactivity found in either of the ELISA tests. One person, aged 45 years, had the classic signs of elephantiasis of the lower left leg and had been treated several times with diethylcarbamazine. This patients had no microfilariae, but was positive on the IgG4 and the 0g4C3 ELISA's.

Discussion

The major concern raised by this study was the number of positive cases of Bancroftian filariasis not detected using the presence of microfilaraemia alone to make the diagnosis. About twice the number of those found positive for microfilaraemia were actually found to be infected, as indicated by the prevalence of antigenaemia and filarial specific IgG4 antibody. There could be several reasons for this disparity; the circulating microfilariae are present in such low density that they are below the 1 mf/ml detection limit of the Knott test or the infection could be prepatant with the *W. bancrofti* adult worms not yet producing mf or the infection could be occult with *W. bancrofti* that have stopped producing mf.

The IgG4 ELISA failed to detect 5% of those found positive for microfilariae. This group probably represents early filarial infections. Detectable levels of IgG4 antibodies are found in the chronic phase of the disease and would possibly not be detectable in early infections. Even though the IgG4 test produced some false negatives the overall prevalence in the three communities was similar to that found in the antigen capture assay. The antigen capture ELISA was found to be positive in all cases of microfilaraemia.

Clinical signs showed no consistent trends when compared to the diagnostic tests being screened in this study. Clinical observations, especially lymphadenopathy, will always be difficult to attribute wholly to lymphatic filariasis.

The increasing prevalence with age in the two serological assays, when compared to microfilaraemia, indicates a constant acquisition of long lived adult worms in the community compared to the constant loss of the relatively short lived microfilariae.

The inability to diagnose all cases of filariasis by detecting microfilariae alone is of concern. The World Health Organization recently estimated that 80 to 90 million people are infected with *W. bancrofti*. The results from this study suggest that this figure could possibly be greater than 200 million. Intuitively the antigen capture ELISA test would appear to be detecting all infections. However this can not be confirmed. Diagnostic tests such as the two serological tests screened here need to be developed into simple, inexpensive field kits for use in epidemiological surveys and disease control work in filarial infected areas.

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References

Department of Health, Papua New Guinea: Papua New Guinea National Health Plan 1991–1995. Depart of Health (1991)

Forsyth, K.: New approaches to the control of lymphatic filariasis using diethylcarbamazine. Papua New Guinea. Med. J. 30 (1987) 189–191

Kazura, J. W., R. Spark, K. Forsyth, G. Brown, P. Heywood, P. Peters, M. Alpers: Parasitologic and clinical features of Bancroftian filariasis in a community in East Sepik Province, Papua New Guinea. Am. J. Trop. Med. Hyg. 33 (1984) 1119–1123

Knight, R., K. P. W. J. McAdam, Y. G. Matola, V. Kirkham: Bancroftian filariasis and other parasitic infections in the Middle Fly River region of Western Papua New Guinea. Annal. Trop. Med. Parasitol. 76 (1979) 565–576

Kwan-Lim, G., K. P. Forsyth, R. M. Maizels: Filarial-specific IgG4 response correlates with active *Wuchereria bancrofti* infection. J. Immunol. 145 (1990) 4298–4305

Lal, R. B., E. A. Ottesen: Enhanced diagnostic specificity in human filariasis by IgG4 antibody assessment. J. Infect. Dis. 158 (1988) 1034–1037

Manson, P.: Medical report, China Imperial Maritime Customs, Shanghai. 18 (1880) 36–39

Manson-Bahr, P. E. C., D. R. Bell: Examination of blood for presence of microfilariae. Mansons Tropical Diseases 19 (1987) 1494

More, S. J., D. B. Copeman: A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. Trop. Med. Parasitol. 41 (1990) 403–406

Schuurkamp, G. J. T., M. Matango, R. Kereu, J. Napil: Malaria, splenomegaly and filariasis in the Ok Tedi area of the star mountains, Papua New Guinea, Three years after residual DDT spraying. Papua New Guinea Med. J. 30 (1987) 291–300

Turner, P. F.: Rumginae report. Anton Breinl Centre for Tropical Health and Medicine (1991)

Dr. Paul Turner

Anton Breinl Centre
James Cook University of North Queensland
Townsville
Australia