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The significance of guinea worm infection in the immunological diagnosis of onchocerciasis and bancroftian filariasis

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Abstract

Infections with *Dracunculus medinensis* frequently occur in the same geographical area as infections with *Onchocerca volvulus* and *Wuchereria bancrofti*. This study analysed the significance of *D. medinensis* infections for the specificity and sensitivity of available tests for antibody-based diagnosis of onchocerciasis (using individual recombinant clones OV-10, OV-11 and OV-16, and the OV-7/OV-10/OV-16 tri-cocktail, in an enzyme-linked immunosorbent assay) and for circulating antigen-based diagnosis of bancroftian filariasis (using the TroBio™ and the ICT™ card tests). Some immunological cross-reactivity was observed with all tests. When using individual recombinant *O. volvulus* antigens, the highest assay indices were obtained for clone OV-10, and the lowest for clone OV-16. Testing the serum responses against the tri-cocktail of recombinant antigens did not notably improve the assay indices. Two of 40 serum samples from individuals with patent dracunculiasis gave a false positive response in the ICT™ test and one of these was also positive in the TropBio™ test. Possible implications of applying these diagnostic assays in areas endemic for dracunculiasis are discussed.

Keywords: dracunculiasis, guinea worm infection, *Dracunculus medinensis*, onchocerciasis, *Onchocerca volvulus*, filariasis, *Wuchereria bancrofti*, immunodiagnosis

Introduction

Significant advances have been made in attempts to develop specific and sensitive immunodiagnostic tools for infections with *Onchocerca volvulus* and *Wuchereria bancrofti*. Many recombinant *O. volvulus* antigens have been developed and tested in enzyme-linked immunosorbent assays (ELISAs) (RAMACHANDRAN, 1993). This resulted in the identification of highly specific antigens, although no individual antigen showed 100% sensitivity because of heterogeneity in the response of individuals infected with *O. volvulus*. However, a 'cocktail' of the 3 most promising antigens (OV-7, OV-11 and OV-16), had a specificity of 100% and a sensitivity of 96% (BRADLEY *et al.*, 1993).

For infections with *W. bancrofti*, 2 immunodiagnostic tools based on detection of specific circulating antigens captured by monoclonal antibodies have been developed and marketed commercially, namely the TroBio™ assay based on the monoclonal antibody Og4C3 (MORE & COPEMAN, 1990), and the ICT™ filariasis card test based on the monoclonal antibody AD12.1 (WEIL *et al.*, 1987). Both of these tests have shown sensitivities and specificities close to 100% (WEIL *et al.*, 1987, 1997; TURNER *et al.*, 1993; CHANTEAU *et al.*, 1994a, 1994b; ROCHA *et al.*, 1996; NICOLAS, 1997).

Infections with *D. medinensis* co-exist with *O. volvulus* and *W. bancrofti* infections in many parts of Africa (MCMAHON & SIMONSEN, 1996). However, the significance of infections with *D. medinensis* in the immunodiagnosis of onchocerciasis or bancroftian filariasis when using the newly developed tools has not previously been determined. The present study investigated the extent to which serum antibodies from individuals infected with *D. medinensis* cross-reacted with individual recombinant *O. volvulus* antigens from clones OV-10, OV-11 and OV-16 (LOBOS *et al.*, 1990, 1991; BRADLEY *et al.*, 1991) and with a tri-cocktail of recombinant *O. volvulus* antigens from clones OV-7, OV-11 and OV-16 (RAMACHANDRAN, 1993). Furthermore, the study examined the extent to which the sera cross-reacted in the TroBio™ and ICT™ tests for circulating *W. bancrofti* antigens. Serum samples were obtained from individuals living in an area of northern Ghana which is highly endemic for *D. medinensis* infection. The individuals were categorized as having prepatent or patent *D. medinensis* infections on the basis of long-term clinical and parasitological examinations. Serum samples from people with patent *O. volvulus* or *W. bancrofti* infections were included as controls.

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Materials and Methods

Characterization of sera

Serum samples were obtained from clinically and parasitologically well-characterized individuals from an area of northern Ghana which is highly endemic for *D. medinensis* infection. Procedures and criteria for identification of the study individuals and methods for collection of venous blood and preparation of serum have been described elsewhere (BLOCH & SIMONSEN, in press). Two categories of individuals were included: 9 individuals (4 males and 5 females; mean age 29 years, range 22–47) who did not have a patent *D. medinensis* infection at the time of blood sampling but who developed a patent infection within 4–12 months thereafter (category a: *D. medinensis*, prepatent), and 40 individuals (20 males and 20 females; mean age 31 years, range 8–52) who had a patent *D. medinensis* infection at the time of blood sampling (category b: *D. medinensis*, patent). No transmission of filarial parasites has been recorded in this area, and skin snip and night blood examinations for microfilariae indicated that these individuals were not suffering from onchocerciasis or lymphatic filariasis. Examination of stool and urine for helminth infections indicated that infections with hookworm (70%) and *Strongyloides stercoralis* (10%) were common.

Control sera were collected from individuals living in areas where no transmission of *D. medinensis* occurs. They comprised sera from 10 individuals (5 males and 5 females from the East Usambara Mountains in Tanga Region, Tanzania, where no transmission of *W. bancrofti* takes place; mean age 32 years, range 16–70) who had microfilariae of *O. volvulus* in a skin snip (category c: *O. volvulus*), 10 individuals (5 males and 5 females from a highly endemic coastal area of Tanga Region, where no transmission of *O. volvulus* takes place; mean age 37 years, range 20–55) who had microfilariae of *W. bancrofti* in their blood (category d: *W. bancrofti*), and 5 Danish individuals (3 males and 2 females; mean age 26 years, range 21–33) who had never been to a tropical country (category e: non-endemic control).

Responses to O. volvulus *recombinant antigens*

The serum immunoglobulin G (IgG) antibody reactivities to individual recombinant antigens (OV-10, OV-11 and OV-16) were analysed by ELISA at the National Institutes of Health, Bethesda, Maryland, USA, according to standard procedures (LOBOS *et al.*, 1991; BRADLEY *et al.*, 1993). The reactivity was graded as – (less than the mean plus 2 SD of the absorbance values for sera from the non-endemic control category e); ± (mean plus 2–3 SD for category e); + (mean plus 3–4 SD for category e), ++ (mean plus 4–5 SD for category e); +++ (mean plus 5–6 SD for category e) and ++++ (above mean plus 6 SD for category e). A response was defined as positive if it were + or greater.

The serum IgG antibody to the antigen tri-cocktail (OV-7, OV-11 and OV-16) was analysed by ELISA at the Swiss Tropical Institute, Basel, Switzerland, according to standard procedures (SPEISER, 1982; KARAM & WEISS, 1985). A response was defined as positive if it was equal to or above the mean plus 2 SD of the absorbance values for sera from the non-endemic control category e.

Circulating W. bancrofti *antigens*

Sera were examined for circulating *W. bancrofti* antigen by the TropBio™ test (JCU Tropical Biotechnology, Australia) and the ICT™ filariasis card test (ICT Diagnostics, New South Wales, Australia) by following instructions from the manufacturers. The TropBio™ test was performed on serum specimens after pretreatment by boiling, and all sera were tested in duplicate. Specimens with more than 32 antigen units on average (≥ titre group 3) were considered positive. The ICT™ test was performed on serum specimens without pretreatment, and the results were read after 10–15 min.

Results

Responses to individual O. volvulus *recombinant antigens*

Sera from 20 individuals with patent *D. medinensis* infection (category b), 10 individuals with *O. volvulus* infection (category c), 10 individuals with *W. bancrofti* infection (category d) and 5 non-endemic controls (category e) were tested against individual *O. volvulus* antigens from the clones OV-10, OV11 and OV-16 (Table 1).

Data from the subjects with *D. medinensis* and *O. volvulus* infection were used to estimate sensitivities and specificities of the tests for the diagnosis of *O. volvulus* infection. Taking a response of + as the threshold for a positive response, the sensitivities were 100% for each of the 3 antigens and the specificities were 60%, 45% and 40% for OV-10, OV-11 and OV-16, respectively. Increasing the threshold level to ++ and +++ resulted in a progressive reduction in sensitivity and increase in specificity. Thus, when using +++ as the lowest positive response, the sensitivities were 80%, 60% and 70%, and the specificities were 100%, 95% and 95%, for OV-10, OV-11 and OV-16, respectively.

Responses to the O. volvulus *antigen tri-cocktail*

All sera were tested with the *O. volvulus* antigen tri-cocktail (clones OV-7, OV-11 and OV-16 combined) (Table 2). None of the sera from individuals with a prepatent *D. medinensis* infection and 2 of the sera (5%) from those with a patent *D. medinensis* infection gave a positive response to the tri-cocktail. Furthermore, one of the sera (10%) from individuals infected with *W. bancrofti* and 8 of the sera (80%) from those infected with *O. volvulus* responded positively. None of the sera from non-endemic control individuals responded to the tri-cocktail. On the basis of the results from individuals with patent *D. medinensi* and *O. volvulus* infections only, the assay sensitivity was 80% and the specificity was 95% for diagnosis of *O. volvulus* infection.

Table 2. Antibody responses to the *Onchocerca volvulus* antigen tri-cocktail (clones OV-7, OV-11 and OV-16) of sera from individuals with various infections and from uninfected control individuals

Response category	No. of sera, Infection category[a] a	b	c	d	e
Negative	9	38	2	9	5
Positive	–	2	8	1	–
Total	9	40	10	10	5

[a]Infection categories: a, prepatent *Dracunculus medinensis* infection; b, patent *D. medinensis* infection; c, microfilaridermic *Onchocerca volvulus* infection; d, microfilaraemic *Wuchereria bancrofti* infection; e, control subjects from a non-endemic region.

Table 1. Graded antibody responses to recombinant *Onchocerca volvulus* antigens from clones OV-10, OV-11 and OV-16 of sera from individuals with various infections and from uninfected control individuals

Response category	No. of sera, OV-10 Infection category[a] b	c	d	e	OV-11 Infection category[a] b	c	d	e	OV-16 Infection category[a] b	c	d	e
–	10	–	6	5	8	–	4	5	7	–	3	5
±	2	–	1	–	1	–	1	–	1	–	–	–
+	7	1	2	–	9	–	2	–	8	1	4	–
++	1	1	–	–	1	4	2	–	3	2	2	–
+++	–	7	1	–	1	1	1	–	1	1	1	–
++++	–	1	–	–	–	5	–	–	–	6	–	–
Total	20	10	10	5	20	10	10	5	20	10	10	5

[a]Infection categories: b, patent *D. medinensis* infection; c, microfilaridermic *Onchocerca volvulus* infection; d, microfilaraemic *Wuchereria bancrofti* infection; e, control subjects from a non-endemic region.

For individuals with a patent *D. medinensis* infection, 8 (40%), 11 (55%) and 12 (60%) responded positively to OV-10, OV-11 and OV-16, respectively. Among the individuals with a *W. bancrofti* infection, 3 (30%), 5 (50%) and 7 (70%) responded positively to OV-10, OV-11 and OV-16, respectively. All sera from individuals infected with *O. volvulus* responded positively to all 3 antigens, and the response was generally stronger with these sera than with positive sera from individuals with *D. medinensis* or *W. bancrofti* infection. None of the endemic control sera responded to any of the 3 antigens.

Tests for circulating W. bancrofti *antigen*

All sera were tested for circulating *W. bancrofti* antigen with the TropBio™ (Table 3) and ICT™ (Table 4) tests. Sera from individuals infected with *W. bancrofti* all gave positive results for circulating antigens in both tests. Among individuals with patent *D. medinensis* in-

Table 3. Results of the TropBio™ test for circulating *Wuchereria bancrofti* antigen with sera from individuals with various infections and from uninfected control individuals

Response category		No. of sera Infection category[a]				
Antigen units	Titre group	a	b	c	d	e
<10	1	9	24	–	–	5
10–32	2	–	15	10	–	–
33–128	3	–	–	–	–	–
129–512	4	–	1	–	–	–
513–2048	5	–	–	–	–	–
2049–8192	6	–	–	–	2	–
8193–32000	7	–	–	–	–	–
>32000	8	–	–	–	8	–
Total		9	40	10	10	5

[a]Infection categories: a, prepatent *Dracunculus medinensis* infection; b, patent *D. medinensis* infection; c, microfilaridermic *Onchocerca volvulus* infection; d, microfilaraemic *Wuchereria bancrofti* infection; e, control subjects from a non-endemic region.

Table 4. Results of ICT™ test for circulating *Wuchereria bancrofti* antigen with sera from individuals with various infections and from uninfected control individuals

Response category	No. of sera Infection category[a] a	b	c	d	e
Negative	9	38	10	–	5
Positive	–	2	–	10	–
Total	9	40	10	10	5

[a]Infection categories: a, prepatent *Dracunculus medinensis* infection; b, patent *D. medinensis* infection; c, microfilaridermic *Onchocerca volvulus* infection; d, microfilaraemic *Wuchereria bancrofti* infection; e, control subjects from a non-endemic region.

fection, 2 were positive in the ICT™ test and one of these was also positive in the TropBio™ test. None of the sera from individuals with prepatent *D. medinensis* infection or patent *O. volvulus* infection, or from non-endemic control individuals, was positive in either test. On the basis of the results obtained from individuals with patent *D. medinensis* and *W. bancrofti* infections, the assay sensitivities were 100% and 100%, and the specificities were 97·5% and 95%, for the diagnosis of *W. bancrofti* infection in the TropBio™ and ICT™ tests, respectively.

Discussion

The present study assessed the implications of infections with *D. medinensis* in the immunodiagnostic assays already developed for infections with *O. volvulus* and *W. bancrofti*. Considering that *D. medinensis* co-exists with *O. volvulus* and *W. bancrofti* in many parts of West and Central Africa (MCMAHON & SIMONSEN, 1996), it seems relevant to take infections with *D. medinensis* into account when developing immunodiagnostic tools for infections with *O. volvulus* or *W. bancrofti*. Furthermore, extensive immunological cross-reactivity between *D. medinensis* antigens and sera from individuals infected with *O. volvulus* has previously been observed (FAGBEMI & HILLYER, 1990; GARATE *et al.*, 1990; BLOCH *et al.*, 1993).

Some immunological cross-reactivity of sera from persons infected with *D. medinensis* was observed in all the tests. The highest assay indices in response to individual *O. volvulus* antigens were obtained with OV-10 and the lowest with clone OV-16, although the differences were not large. Assay indices varied with the cut-off level applied, with lower sensitivities and higher specificities resulting from an increase in cut-off level. The recommended cut-off level for an immunodiagnostic test depends on the purpose of the test (RAMACHANDRAN, 1993), and for a diagnostic test developed for epidemiological studies and for the monitoring of disease control programmes it has been argued that specificity should be given higher priority than sensitivity (BRADLEY *et al.*, 1993), because the emphasis is on measuring the level of infection in a human population rather than on individual diagnosis.

The *O. volvulus* antigen tri-cocktail consists of 3 recombinant antigens (OV-7, OV-11 and OV-16) which are very specific when tested individually against sera from individuals infected with *O. volvulus* or *W. bancrofti* (see RAMACHANDRAN, 1993). This tri-cocktail, as well as cocktails of other recombinant *O. volvulus* antigens, was prepared in an attempt to develop an immunodiagnostic test for infection with *O. volvulus* which is not only specific, but also highly sensitive (BRADLEY *et al.*, 1993). Clone OV-7 was included in the tri-cocktail instead of clone OV-10, but these 2 antigens are almost identical (LUSTIGMAN *et al.*, 1991; BRADLEY *et al.*, 1993; RAMACHANDRAN, 1993). In the present study, the assay indices were not improved by using the tri-cocktail instead of the individual antigens, showing the importance of also taking dracunculiasis into consideration when evaluating the usefulness of the test for areas of Africa where infection with *D. medinensis* occurs.

Contrary to a previous report (BRADLEY *et al.*, 1993), some control sera from individuals infected with *W. bancrofti* responded to the 3 individual recombinant antigens as well as to the tri-cocktail. This discrepancy may reflect differences in the level of transmission of bancroftian filariasis: BRADLEY *et al.* (1993) obtained sera from the Philippines and Sri Lanka, where the level of transmission is low, whereas sera for the present study were obtained from a highly endemic coastal area of Tanzania (SIMONSEN *et al.*, 1995).

Sera from individuals infected with *D. medinensis* were tested in the TropBio™ and ICT™ assays for circulating *W. bancrofti* antigens. These tests have previously been shown to have high sensitivity for *W. bancrofti* infection, and high specificity in relation to other types of infection (WEIL *et al.*, 1987, 1997; MORE & COPEMAN, 1990). One of the 40 sera from patent dracunculiasis patients tested gave a positive result in the TropBio™ assay and 2 did so in the ICT™ assay. Repeated testing of the positive sera gave the same results. It appears highly unlikely that the individuals (2 males aged 30 and 38 years, respectively) from whom the positive serum samples were obtained were infected with *W. bancrofti*. Both persons were amicrofilaraemic in night blood specimens, presented no sign of lymphatic filariasis, lived in an area of northern Ghana which is not endemic for bancroftian filariasis, had always lived in the same village, and did not travel much. The findings indicate that infections with *D. medinensis* do affect the specificity of the TropBio™ and ICT™ assays, although to a very limited extent.

Despite the use of relatively few sera, our findings indicated that *D. medinensis* infections had some, but not

a major, impact on the results of previously developed immunodiagnostic assays for *O. volvulus* and *W. bancrofti* The present study has, furthermore, contributed qualitative information on the assays which, together with equivalent information obtained for other relevant parasitic infections, may be used to define cut-off levels of general applicability.

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