

ORIGINAL ARTICLE

The schistosoma-specific antibody response after treatment in non-immune travellers

LIV MARIE DUUS¹, ANDERS VITTRUP CHRISTENSEN¹, DORTE NAVNTOFT¹,
BRITTA TARP², HENRIK V. NIELSEN³ & ESKILD PETERSEN¹

From the ¹Department of Infectious Diseases, Aarhus University Hospital, Skejby, Aarhus,
²Department of Infectious Diseases, Regionshospitalet Silkeborg, Silkeborg, and ³Laboratory of Parasitology,
Statens Serum Institute, Copenhagen, Denmark

Abstract

Egg detection is the gold standard in diagnosing and controlling treatment in schistosomiasis, but sensitivity is poor in lightly infected individuals, whereas Schistosoma-specific antibodies are more sensitive. The purpose of the study was to evaluate use of Gut Associated Antigen (GAA) and Membrane Bound Antigen (MBA) assays in assessment of treatment efficacy and number of treated non-immune individuals with signs of treatment failure. In a retrospective study, residents in Denmark diagnosed with positive Schistosoma antibodies in the period 1987 – 2004 were offered follow-up including analyses for GAA, MBA, IgE and eosinophil count. Among 98 patients with positive antibody at time of diagnosis, 73 were examined for eggs and 27% had detectable eggs. 15% still had detectable living eggs after 1 course of treatment. At follow-up it was demonstrated that antibodies continued to increase for up to 6 months after treatment and average duration of positive GAA antibody was approximately 10 y. The study confirms that the GAA- and MBA-IFAT are not suitable in monitoring results of therapy. Treatment failure in 15% of non-immune patients indicates that studies are needed to define the correct dose of praziquantel in those individuals or to evaluate if resistance to praziquantel is a growing problem.

Introduction

Worldwide, schistosomiasis remains 1 of the most prevalent parasitic diseases. Due to successful control programmes, the distribution of schistosomiasis has changed over the last 50 y, but the number of people estimated to be infected or at risk of infection has not been reduced [1,2].

In non-endemic areas the numbers of imported cases with schistosomiasis are increasing due to tourism and immigration from endemic areas, and most imported infections in travellers from Europe are acquired in tropical Africa [3,4]. Acute infection may cause an acute febrile reaction with eosinophilia, abdominal pain and cough [5–7], but very often the infection is asymptomatic until late stages of the infection. Severe sequelae due to schistosomiasis occur rarely in travellers with short-term exposure [8,9]. However, for immigrants coming to non-

endemic countries the diagnosis of schistosomiasis is crucial, as are proper treatment and follow-up to avoid chronic manifestations with irreversible fibrosis or malignancy.

Detection of eggs in faeces, urine or biopsies is the gold standard of diagnosing or controlling the response to treatment in cases of schistosomiasis, but in lightly infected individuals with low worm burden, the sensitivity of this test is poor [10]. In such situations detection of Schistosoma-specific antibodies is more sensitive, including the immunofluorescence antibody test (IFAT). Due to this, the use of indirect approaches is widespread in non-endemic areas [11–14].

In a previous study, we suggested the use of assay of specific antibodies against GAA in individuals from non-endemic areas and against both GAA (Gut Associated Antigen) and MBA (Membrane Bound

Correspondence: E. Petersen, Department of Infectious Diseases, Aarhus University Hospital, Skejby, DK-8200 Aarhus N, Denmark. (Tel: +45 89498307. Fax: +45 89498360. E-mail: eskildp@dadlnet.dk)

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Antigen) in individuals from schistosomiasis-endemic areas in order to diagnose schistosomiasis [14]. However, using the GAA- and the MBA-IFAT to monitor the results of therapy is suboptimal as the antibody level has a tendency to rise after chemotherapeutic treatment, and may remain elevated for more than 1 y [15,16]. Meanwhile, for how long a time the antibody level might remain elevated, despite successful treatment, is unknown.

The purpose of our study was to evaluate the use of specific antibodies against GAA and MBA assays in the assessment of treatment efficacy and the number of treated non-immune individuals with signs of treatment failure.

Methods and materials

Patients

In a retrospective study residents in Aarhus County diagnosed with a positive *Schistosoma* antibody assay in the period 1987 – 2004 were identified from the archives at the Laboratory of Parasitology, Statens Serum Institute, Copenhagen, Denmark. These individuals were offered a follow-up visit at the Department of Infectious Diseases, Aarhus University Hospital. At the visit they were interviewed concerning their travel history prior to the diagnosis of schistosomiasis, their previous examinations and treatment for schistosomiasis. Their medical records were reviewed concerning the results of the previous examinations: microscopy for eggs and/or serology. Moreover, at the follow-up visit their *Schistosoma* serology, IgE status and eosinophil count were measured. Individuals with a positive serology at follow-up were offered a new examination for eggs in stool, urine or in rectal biopsies.

The examination of stool, urine, rectal biopsies and the immunofluorescence antibody test (IFAT) against schistosoma species were performed as previously described [13,14]. The diagnosis of schistosomiasis was based on a positive serology (GAA and/or MBA) and/or positive egg detection in stool, urine or rectal biopsy.

Treatment history

At the time of diagnosis the patients were treated with a single dose of 40 mg/kg praziquantel for *S. haematobium* and 2 doses of 40 mg/kg given 4 h apart for *S. mansoni*.

Patients with signs of schistosomiasis at follow-up received, according to the guidelines, 1 course

of treatment. Some patients received more than 1 treatment.

Data analysis

The serological endpoint dilution steps were used instead of the reciprocal dilution for the presentation of the data to give equal weight to each dilution step. Two-fold dilutions starting at 1: 16 up to 1: 256 were used throughout, equivalent to dilution step 1 to 5. Regression analysis was made for each patient course and used to determine the mean decrease in antibody levels over time.

Results

Study population

98 individuals (56F, 42M) with a mean age of 26.7 y (range 6 – 60 y) were included. All the individuals had serology-proven schistosomiasis and a relevant travel history to endemic areas.

Travel history

Information on destinations was available from all 98 individuals: 93 had visited tropical Africa, 3 North Africa and 2 Asia. The median duration of the travel prior to diagnosis was 6 months (range 0.5 – 120; $n = 90$). For 8 individuals we did not know the duration of travel.

Detection of *Schistosoma* eggs in faeces, urine and rectal biopsies

At time of diagnosis 73 of the 98 patients were examined for *Schistosoma* eggs in rectal biopsies, stool and/or urine samples. 58 patients had a sample taken from more than 1 location (stool/urine/rectal mucosa). A total of 163 samples were examined: 69 stool samples, 55 urine samples and 39 rectal mucosa biopsies. Eggs were found in 2/69 (2.9%) of the stool samples, 5/55 (9.1%) of the urine samples and 15/39 (38.5%) of the rectal mucosa biopsies. Among the 73 patients examined for eggs, only 20 (27.4%) were found positive.

Schistosoma species could be classified in 16 out of the 20 patients with positive egg tests (Table I). Three of 20 patients (15%) still had detectable living eggs after 1 course of treatment at follow-up after 4 months, 1 y and 3 y, respectively.

The mean of the highest detected GAA/MBA dilution step value in the egg-positive group was 3.8 (95% CI 3.5 – 4.1), and 3.7 (95% CI 3.4 – 4.0) in

Table I. Species classification from the 20 patients tested positive for eggs, categorized by type of sample material.

	<i>S. haematobium</i> (<i>n</i> = 7)	<i>S. mansoni</i> (<i>n</i> = 9)	Unclassified (<i>n</i> = 4)
Rectal mucosa biopsy	1 ^a	8 ^c	4
Stool sample	1	1	-
Urine sample	3 ^b	-	-
Rectal mucosa biopsy and urine	2	-	-

^a1 patient: no faeces sample was examined.

^b2 patients: no rectal mucosa biopsy was examined.

^c2 patients: no urine sample was examined.

the egg-negative group. The difference was not statistically significant ($p = 0.68$).

Eosinophilia and total IgE

At time of diagnosis 39/98 patients were tested for eosinophilia (normal range $<0.5 \times 10^9/l$) and all 39 patients were examined for eggs. 17 of these 39 individuals (43.6%) had an increased eosinophil count and in 7 (41.2%) of these patients eggs were detected. Five of the 22 patients (22.7%) with a normal eosinophil count had detectable eggs. Odds ratio for a positive egg test if increased eosinophil count was 2.4 (95% CI 0.6 – 9.5). At the time of diagnosis total IgE was measured in only 5 patients and 2 were found with elevated level (normal range <150 IU/l).

At follow-up all 98 patients were examined for eosinophilia and an increased eosinophil count was

measured in 22 patients (21%). IgE were measured in 90 of the 98 patients and only 10 of these had elevated levels.

Detection of specific antibodies to Schistosoma antigens

All patients were tested positive for either GAA or MBA. 30 of the 98 patients were tested positive for MBA. 16 of these were positive in both GAA and MBA. Seven of the 16 had a positive GAA titre value measured prior to a positive MBA; 7 had their first positive MBA and GAA measured at the same time, and 2 became positive in MBA before GAA. 14 patients were found not positive for GAA at any time. Consecutive antibody analysis was only performed in 56 patients, who were all treated at least once with praziquantel and these were included in the analysis of the long-term development of antibody levels after treatment.

After diagnosis and treatment, subsequent antibody measurements showed that the GAA antibody titre continued to increase during the first 6 months after diagnosis, with a peak between 4 and 6 months (Figure 1).

The mean GAA titre decreased slowly after treatment, as shown in Figure 2. The logistic regression line reached zero after approximately 10 y.

28 patients lost specific antibodies during the observation period and the range of time until first negative serology test was 1.7 to 15.8 y. Five patients followed for more than 10 y never showed a negative antibody test despite being treated according to guidelines.

Data were analysed for the length of stay at Schistosoma endemic areas for 1 y or more ($n = 24$)

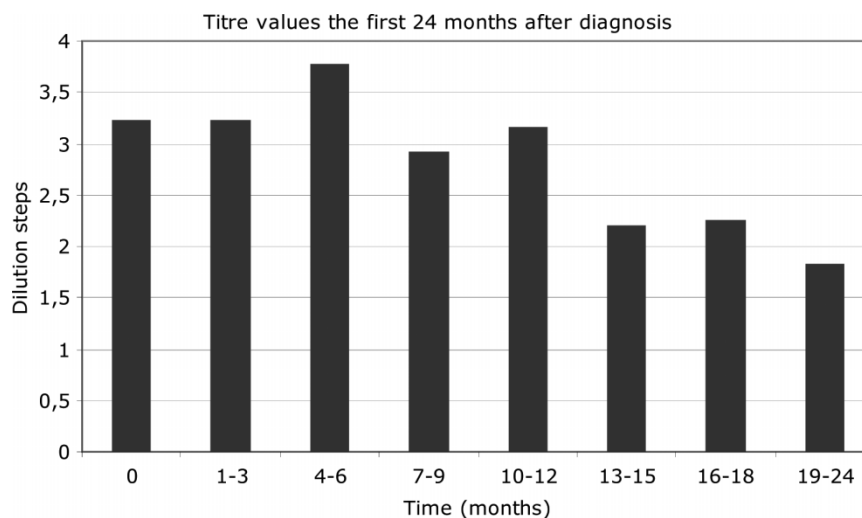


Figure 1. Average values of anti-GAA separated quarterly for the 34 patients with more than 1 value within the first 2 y after first contact with the hospital.

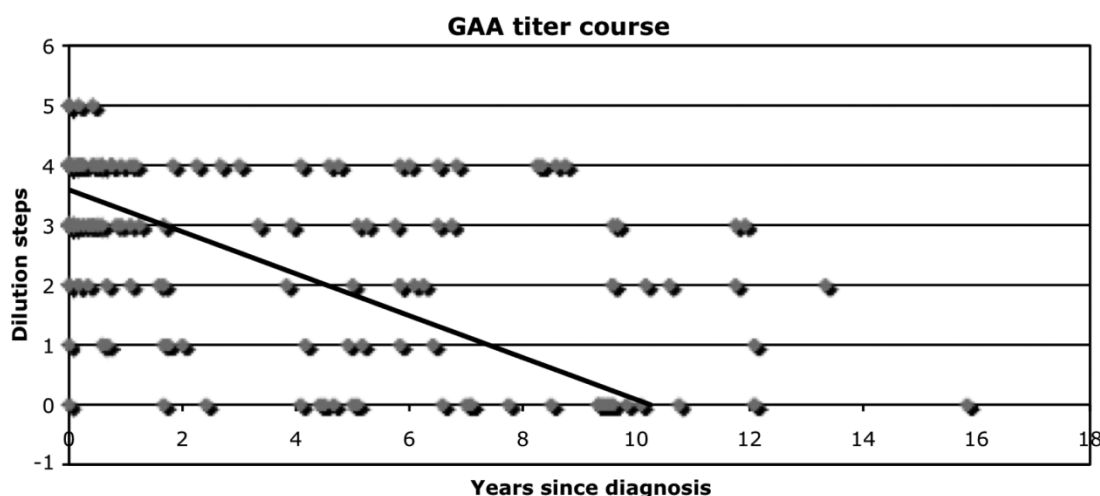


Figure 2. The dots represent all the measured GAA values from the patients. For each patient the slope and interception are calculated.

or less than 1 y ($n=32$). The change over time in GAA antibody levels was compared in patients treated only once ($n=44$) with patients treated 2 times or more ($n=12$). The mean slope and interception was found in the different groups (Table II), but there was no statistically significant difference.

Discussion

In most cases travellers infected with *Schistosoma* species are asymptomatic and suspicion of infection is often based on a history of contact with fresh water in schistosomiasis endemic areas [6].

The traditional and gold standard diagnostic test for infection with *Schistosoma* spp. is detection of egg in stool, urine and/or rectal biopsies. Meanwhile, the worm burden is often low in travellers exposed for only a short period and the excretion of eggs is usually below the detection level [14,17]. In this retrospective study we found *Schistosoma* eggs in only 27.4% (20/73) of travellers with a positive *Schistosoma*-specific antibody test and the most sensitive method was examination of rectal biopsies,

which is in accordance with a previous study of schistosomiasis in travellers [18]. This indicates that egg detection is not a sensitive method for diagnosing schistosomiasis in a non-immune population. The retrospective character of the study and the clinical assessment at the time of diagnosis explain why not all patients were examined for egg.

Other methods of diagnosing schistosomiasis include the detection of circulating antigen especially the circulating anodic antigen [19], but the sensitivity is low in patients with a low or undetectable egg count [20]. Repeated measurements of circulating anodic antigen and circulating cathodic antigen is a valuable method for assessing treatment efficacy in populations living in *Schistosoma* highly endemic areas [17,21], as their presence may indicate active infection in that population while they showed a sensitivity of only 23%–29% in a population from a low endemic area [22]. Experience with specific antibodies after treatment is limited [23]. A study from the UK followed patients for 1 y after treatment and reported that antibody titres had increased in 16%, was the same as baseline in 23%, and reduced in 61% of the patients [24]. A study

Table II. Slope and interception was found for each patient via regression of their GAA course.

No. treatments	No. patients	Mean interception	95% CI	Mean slope	95% CI
1	44	3.62	(3.43–3.81)	–0.38	(–0.49–0.28)
2–3	12	3.47	(2.91–4.02)	–0.22	(–0.33–0.11)
<i>t</i> -test		* $p=0.52$		* $p=0.16$	
Exposure time					
<1 y	32	3.65	(3.42–3.87)	–0.38	(–0.49–0.27)
>1 y	24	3.49	(3.16–3.82)	–0.32	(–0.48–0.17)
<i>t</i> -test		* $p=0.40$		* $p=0.63$	
All patients	56	3.59	(3.40–3.77)	–0.35	(–0.44–0.26)

Tests for significant difference of mean slope (dilution steps/y) and mean interception (of the y-axis) between groups categorized according to the number of treatments and exposure time.

from Sweden followed patients for 2 y after treatment and found that 40/42 were GAA-positive at follow-up [25]. Detection of Schistosoma-specific antibodies by immunofluorescence has been shown to be a sensitive method of diagnosing schistosomiasis in non-immune patients [13,14]. In the present study, we did not find any difference in the titre of Schistosoma-specific antibodies between egg-positive and egg-negative individuals. This may indicate that a strong antibody response is elicited even though the worm load is so low that it is not possible to detect eggs in clinical samples.

Although the presence of GAA antibodies normally is regarded as an early sign of infection [13,14], in the present study GAA antibodies were preceded by MBA antibodies in 7 individuals. Moreover, 14 subjects failed to develop antibodies to GAA. Owing to this, it seems reasonable to measure antibodies to both GAA and MBA in the diagnostics of Schistosoma infection in travellers from non-endemic areas.

Our finding of a continuing increase in levels of GAA antibodies up to 6 months after treatment might be a consequence of boosting of the immune system as a result of the destruction of parasites (Figure 1).

It can be argued that the long-term persistence of antibodies seen in our patient population is an indication of treatment failure. We do not believe that this is the whole explanation as it would have resulted in an increased fraction of patients excreting eggs or showing elevated total IgE or eosinophilia. Furthermore, it would indicate that standard praziquantel treatment was ineffective in the vast majority of cases. This seems improbable.

The GAA reactivity declined slowly after treatment. Based on the regression lines, it took approximately 10 y before the subjects became serological-negative (Figure 2). Nine per cent of patients (5/56) followed for more than 10 y after diagnosis never showed a negative antibody test. This demonstrates that the antibody response may be long-lasting even without re-exposure and clearly indicates that repeated measurements of GAA IgG antibodies are not suitable as an indicator of successful treatment.

In our study 3 patients still had living eggs after 1 course of treatment with praziquantel, indicating treatment failure. Low efficacy of praziquantel has been reported in a study from Senegal, and in the same study lower cure rates were found in children than adults [19]. The finding that cure rates were lower in children compared to adults indicates that immunity, which increases with age and time of exposure, is acting in synergy with praziquantel. This suggests that treatment effects in Schistosoma

immune patients cannot be directly extrapolated to effects in non-immune travellers.

Only 41% (7/17) of egg-positive individuals had elevated eosinophil counts at baseline. This confirms previous findings [14] indicating that eosinophilia is not a good indicator of schistosomiasis.

In conclusion, by this retrospective study we confirm that the GAA- and MBA-IFAT are not suitable to monitor the results of therapy. However, for the first time a follow-up has been performed in a population of non-immune individuals previously treated for schistosomiasis in order to follow the duration of elevated levels of Schistosoma antibodies. Previously it has been concluded that the antibodies may remain elevated for y after treatment [15,16]. This study shows that the antibody titres may even increase within the first 6 months and in some cases remain elevated for more than 10 y after treatment without re-exposure.

Moreover, treatment failure in 15% of non-immune patients indicates that further studies are needed to define the correct dose of praziquantel in individuals with no prior immunity to Schistosoma spp. or to evaluate if resistance to praziquantel is a growing problem.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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