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Assessment of cure by detection of circulating antigens in serum and urine, following schistosomiasis mass treatment in two villages of the Office du Niger, Mali

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Abstract

Eight weeks after mass chemotherapy with 40 mg/kg praziquantel in two villages in Office du Niger (an irrigation area in Mali, endemic for both *Schistosoma haematobium* and *Schistosoma mansoni*) the circulating anodic (CAA) and cathodic (CCA) antigen detection assays were carried out on serum and urine samples. Both prior and post treatment highest prevalence was measured with the urine-CCA assay. Cure rates determined by antigen detection were almost half that of the egg counting methods. It was shown that the reduction in intensity should be preferentially assessed by the serum-CAA assay. Compared with egg detection, a single antigen detection assay gave a much better assessment of the impact of chemotherapy. © 1997 Elsevier Science B.V.

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1. Introduction

Several studies indicate the lack of sensitivity of parasitological egg counting in diagnosing *Schistosoma* infections, even after repeated egg counts. As a result, prevalences will be underestimated and cure rates overestimated (de Vlas and Gryseels, 1992; de Vlas et al., 1993). A relatively new alternative method is the antigen detection test, which has been used in the diagnosis and the follow-up after anti-schistosomal treatment (de Jonge, 1990; Van Lieshout et al., 1991, 1993; Deelder et al., 1994, Kremsner et al., 1994).

In Mali, the circulating anodic and cathodic antigen detection assays (circulating anodic (CAA) and cathodic (CCA), respectively) were compared with the classical parasitological methods during surveys in different regions of the country. The first results, from samples from two villages in Dogon Country where *Schistosoma haematobium* was the predominant infection, indicated that serum-CAA detection was as effective as urine examination, for determining prevalences of infection in a highly endemic village, and better than parasitological examination in a moderately endemic village (De Clercq et al., 1995). Furthermore, cure rates estimated by the serum-CAA assay 6 weeks after treatment were significantly lower than those determined parasitically, suggesting overestimation of cure by egg counting. In other studies where the two adult worm circulating antigen detection assays were evaluated on pre-treatment urine and serum samples from Office du Niger (a mixed *S. haematobium*/*S. mansoni* endemic area), the highest prevalences were measured with the urine-CCA assay. This assay was also the most sensitive test in the diagnosis of infections by *S. haematobium*, *S. mansoni* or a mixed infection (De Clercq et al., 1997).

In Dogon Country where *S. haematobium* is predominant, cure rates were assessed only with the serum-CAA assay. The objective of the present study was to assess cure rates in a mixed *S. haematobium*/*S. mansoni* endemic area, by applying the two circulating antigen detection assays CAA and CCA on serum and on urine samples.

2. Materials and methods

2.1. Parasitological techniques

Eight weeks after mass treatment with praziquantel (40 mg/kg) in Rigandé and Siguivoucé (two villages of the Office du Niger, which is a vast irrigation area in Mali, with permanent transmission of schistosomiasis), 337 urine, 352 serum and only 134 stool samples could be collected in the two villages. For each individual, 10 ml urine was filtered (Nuclepore), examined microscopically and individuals with

a negative urine egg count were re-examined the following day; one Kato slide (41.6 mg) was prepared per individual and examined microscopically. Only one stool sample could be collected, as there is great reluctance to handle stools, to be seen handing in specimens, as well as frequent constipation due to their rice diet.

2.2. Circulating antigen detection techniques

Circulating anodic (CAA) and cathodic (CCA) antigen levels were determined in the laboratory in Bamako on blood and urine samples. A total of 5 ml of blood (serum separated) and 1 ml of urine were collected and stored at -20°C until use. CAA was determined by ELISA according to Deelder et al. (1989), using an anti-CAA IgG1 monoclonal antibody 120-1B10-A, both as capture antibody and as the alkaline phosphatase-conjugated antigen-detecting antibody (120-1B10-A/AP). Urine samples were incubated at 70°C for 30 min and tested in 2-fold dilution series, starting at dilution 1/1; serum samples were pretreated with an alkaline solution, incubated at 70°C for 30 min and tested in 2-fold dilutions, starting at dilution 1/4. The reciprocal value of the last sample dilution showing absorbance above background level (mean absorbance + 3 S.D. of buffer controls if coefficient of variation $< 5\%$ or + 2 S.D., if coefficient of variation between 5 and 10%) was taken as the titre. Serum samples were considered positive at a titre ≥ 4, and urine samples at a titre ≥ 1. CCA was determined by ELISA according to de Jonge et al. (1990), using an anti-CCA IgG3 mouse monoclonal antibody 54-5C10-A as coating and a biotinylated IgM mouse monoclonal antibody 8-3C10 as the detection antibody. Streptavidin-alkaline phosphatase was used as enzyme label. Serum samples were tested and considered positive as for CAA; urine samples were pretreated with an alkaline solution, incubated during 30 min at 70°C, tested in 2-fold dilution series and considered positive at a titre ≥ 2.

Cure rates and reduction in intensities were calculated in 97 individuals for whom a complete set of results was obtained before and after treatment. Cure rates represent the proportion of individuals positive for eggs (or antigen) before treatment, who became negative 8 weeks after treatment. Intensities of infection were expressed as the geometric mean of positive egg counts or antigen concentrations. Spearman's rank correlations were used to estimate concordance between egg output and the circulating antigen titres.

3. Results

The prevalences of infection and cure rates as determined by parasitological examination of stool and urine and by the circulating antigen detection assays are shown in Table 1. Eight weeks after mass treatment with 40 mg/kg praziquantel, the cure rate as determined by two urine egg counts (87%) and a single stool egg count (96%) was considerable. Before and after treatment, the highest prevalence was measured by the urine-CCA assay. Cure rates determined by antigen detection were almost half that of the egg counting methods.

Table 1
Comparison of the cure rates determined by egg counting and antigen detection, 8 weeks after mass treatment in two villages in Office du Niger

	Prevalence		Cure rate %
	Before treatment % (n)	After treatment % (n)	
S.H	80 (78)	10 (10)	87
S.M	71 (69)	3 (3)	96
Mixed inf.	61 (59)	1 (1)	98
S CAA	76 (74)	53 (51)	30
S CCA	60 (58)	32 (31)	47
U CAA	74 (72)	63 (35)	51
U CCA	95 (92)	78 (76)	18

$n = 97$.
S.H., *Schistosoma haematobium* determined by two urine examinations; S.M., *Schistosoma mansoni* determined by a single stool examination; S CAA, serum CAA assay; S CCA, serum CCA assay; U CAA, urine CAA assay; U CCA, urine CCA assay.

Changes in intensity are shown in Table 2. The reduction in intensity of heavy *S. mansoni* (96–100%) and *S. haematobium* infections (100%), as determined by egg counting were very high in both villages. Before treatment the mean CAA levels

Table 2
Comparison of changes in intensity as determined by urine filtration, stool examination and the circulating antigen detection assays

Test	Villages [a]	Intensity before treatment [b]	Intensity after treatment [b]	Change in intensity (%)
S CAA	R	144.4	12.8	91
	S	103	6.8	93
S CCA	R	35.9	25.3	29
	S	50	44	12
U CAA	R	6.7	1	85
	S	6.8	2.4	65
U CCA	R	399.3	143	64
	S	380	945.7	<0[d]
Urine filtration	R	16	3	81(100) [c]
	S	18	2	89(100) [c]
Stool examination (KATO)	R	64	72	<0[d](96) [c]
	S	78	24	69(100) [c]

[a] R, Rigandé, S, Siguivoucé; [b] geometric mean of positive samples (antigen concentrations in ng/ml, number of eggs per 10 ml urine or per g stool; ( ) [c] % change in heavy infection intensities (i.e. >50 ep 10 ml urine, >100 epg stool); [d] intensity increased after treatment.

Table 3
Correlations between the tests and the urine and stool examination at enrollement

	S CAA	S CCA	U CCA	U CAA	
Rigandé	0.29*	0.38*	0.27*	0.49*	U
	0.17 (ns)	0.58*	0.45*	0.30*	S
Siguivoucé	0.49*	0.32*	0.53*	0.19**	U
	0.44*	0.45*	0.41*	0.41*	S

* Significant $P<0.001$; ** significant $P<0.01$; ns, not significant; U, urine, S, stool.

were much higher than CCA in serum, but lower in urine. Therefore, only serum-CAA and urine-CCA values were used in the remaining analysis. Reduction in intensity, as determined by serum-CAA concentrations, was higher than 90% in both villages. However, the urine-CCA assay presented opposite results for the two villages: a reduction in intensity in Rigandé but an increase in Siguivoucé.

Before treatment, there were significant positive correlations between egg output and the circulating antigen titres, except between serum-CAA and stool examination in Rigandé (Table 3). Very few individuals were found egg positive after treatment and the correlations with antigen titres were not significant.

4. Discussion

Before treatment and 8 weeks after treatment in a mixed *S. haematobium*/*S. mansoni* infection area, the highest prevalences were measured with the urine-CCA assay. The same result was obtained also at 1 and 2 years after treatment in the same villages of the Office du Niger (unpublished results). In our study, where only single stool examinations could be carried out, the true *S. mansoni* prevalence, calculated according to the pocket chart developed by de Vlas et al. (1993), would be 99% instead of the 71% measured; the value of 99% corresponds well with the 95% as measured with the urine-CCA assay. In a recent epidemic outbreak of *S. mansoni* in Northern Senegal (Polman et al., 1995), the prevalence determined by a single urine-CCA assay was even slightly higher than the prevalence determined by repeated faecal egg counts. These results indicate that a single urine-CCA assay is more sensitive than single or repeated parasitological examinations. Its diagnostic performance must also be seen in terms of specificity, however this is much more difficult to measure in an endemic area as 'true negatives' are hard to document.

Cure rates, as determined by egg counting techniques, were very satisfactory (between 87 and 98%) whereas when determined by the circulating antigen assays these rates were quite lower (between 18 and 51%). Similar results were obtained in individuals infected predominantly with *S. haematobium* in another endemic area of the country (De Clercq et al., 1995). The fact that cure rates are overestimated by egg counting techniques is not surprising, given the lack of sensitivity of single egg counts especially at low intensity levels (e.g. after treatment), and the daily

fluctuation of egg excretion. It has been shown that antigen levels in serum vary less during the course of the day and show less day-to-today variation than egg excretion (Deelder et al., 1994). Additionally, recent studies have shown that in urine of *S. mansoni* infected individuals CCA levels, but not CAA levels, were more stable than faecal egg counts (Van Etten et al., 1996).

In contrast with a reinfection study on *S. haematobium* in Cameroon (Kremsner et al., 1994), where CCA levels in serum were the only antigen levels that were not correlated with egg output, in this study CCA levels in serum and in urine correlated with both urinary and intestinal egg excretion. In a previous study (de Jonge et al., 1989) in *S. haematobium* infected patients, urine-CCA levels were not correlated with the number of eggs excreted, whereas in *S. mansoni* infected patients they correlated with egg excretion. The contradictory results obtained with the urine-CCA assay when assessing intensity reduction in the different villages were also observed at 1 and 2 years after treatment (results not shown). There appears to be no explanation for these results. In addition, studies with the urine-CCA assay in schoolchildren from Bamako (Mali) indicated a low sensitivity (about 40%), confirming previous reports that the application of this assay for the diagnosis of *S. haematobium* infections is still controversial (Kremsner et al., 1994). Furthermore, the serum-CAA assay has been shown to be highly specific (Krijger et al., 1994). These results suggest that the serum-CAA assay should be used preferentially to assess the reduction in intensity, as it can be assumed that serum antigen levels reflect worm burdens more directly than those in urine because the latter are probably influenced by renal excretion dynamics.

CAA and CCA have been demonstrated, albeit in low levels, in serum 5 to 6 weeks after exposure in a group of Dutch travellers who were infected with *Schistosoma* while swimming in the Dogon area of Mali (Van Lieshout et al., 1997). The authors postulated that this time period may even be shorter in endemic populations. As 5–6 weeks corresponds with the number of weeks usually employed for assessment of cure (Van Lieshout et al., 1991, 1993), this could hamper the use of circulating antigens to distinguish between failure of treatment and reinfection in areas with continuous high transmission levels. In the present study, reinfection had taken place during the 8 weeks, but at an overall low level, as CAA concentrations in all age groups were lower than 10 ng/ml. Timing of cure rate determination by using antigen detection should take into account the local patterns of transmission.

In conclusion, these results demonstrate the large overestimation of cure as determined after a single stool and two urine examinations in a mixed *S. mansoni*/*S. haematobium* infection area. A single antigen detection assay gave a much better assessment of the impact of mass chemotherapy. The lower cure rates (as determined by antigen detection) could indicate either partial failure of treatment, a temporary inhibition of egg production by the remaining adult worms, or ongoing transmission and reinfection. Failure of praziquantel treatment has, up to now, not been demonstrated in Mali. The postulated temporary inhibition of egg production by adult worms surviving chemotherapy needs further investigation. It is, however, known that the transmission of schistosomiasis in the vast irrigation area under study is permanent throughout the year.

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