

PRE- AND POST-TREATMENT IMMUNODIAGNOSTIC EVALUATION IN HUMAN SCHISTOSOMIASIS MANSONI

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Schistosomiasis control seems to be different in countries where low parasitic burden and asymptomatic clinical patients are the features of majority of cases. Immunological methods must substitute the traditional coprologic techniques used for some decades in the Control Program. Circumoval Precipitin Test (COPT), intradermal test and ELISA with soluble egg antigen (SEA) are evaluated for using as tools for seroepidemiologic studies. COPT and ELISA were performed after treatment to know their utility when impact of chemotherapy must be assessed.

One hundred sixty five persons were followed up 3, 6, 9 and 12 months after treatment. The mean sensitivity of COPT studied by age groups was 95.6% which is very important considering that 88.4% of the studied population excreted less than 100 eggs/gr of feces, while sensitivity of intradermal test was 58.2%. Children showed the highest reactivity to COPT.

*When treatment is effective, COPT reactivity progressively diminish until become negative one year later. In the non cure group, the COPT reactivity diminished but never below 20%. ELISA-SEA did not modify one year after treatment. Effort should be made to isolate fractions of eggs of *Schistosoma mansoni* whose antibodies disappear after treatment.*

Key words: treatment – schistosomiasis – immunodiagnosis

Venezuela is a country with a central coastal focus of schistosomiasis. The transmission and clinical features of the disease seem different from those exhibit by the Brazilian foci.

One of its differential characteristic is the predominance of asymptomatic cases caused by a low parasitic burden (WHO, 1985).

Several large field surveys based on fecal examination, have demonstrated a high prevalence of the disease in the endemic area, specially in Carabobo and Aragua States, with prevalences ranging from 8 to 30% (Aguilar et al., 1986; Balzan & Camejo, 1988). Kato's technique has been the traditional method used for this purpose. In some places, the disease has changed from rural to suburban type of transmission (Alarcón et al., 1987; Balzan & Camejo, 1988).

The sensitivity of stool examination methods diminishes when individuals excrete less than 100 eggs/gr of feces (Mott & Cline, 1980). Therefore, immunological techniques are the diagnostic alternative for areas such as Venezuela and other caribbean countries with low excretion of *Schistosoma mansoni* eggs.

On the other hand, evaluation of the impact of mass chemotherapy is a goal in epidemiologic surveys. In that respect, immunodiagnostic tool for this is desirable. The objective of the present work was to validate various diagnostic immunological techniques when used in our light *S. mansoni* infected patients, as well as to determine the convenient follow up lapse after treatment.

MATERIALS AND METHODS

The chosen community was Caraballeda, a suburban village built around the San Julian River, in the north seashore central Venezuela. After an educative speech to the community, people was interviewed house by house, and the following protocol was carried out:

1. Coprological survey: (a) Two fecal samples collected in different days were examined for each person; (b) Three conventional Kato were performed from each fecal sample, i.e. 6 Kato per person; (c) When a Kato tests was positive, 1 additional Kato-Katz (Katz et al., 1972) was carried out from the fecal sample collected the day of the treatment. Intensity of infection was classified based on the level of eggs elimination as: light, moderate and severe, according to Cook et al. (1974).

2. Before treatment, every patient was clinically evaluated and a blood sample was drawn.

3. An intradermal test (Da Silva et al., 1976) with crude worm antigen (Mayer & Pifano, 1949) was applied to each person. A positive results were considered as any infiltration equal or larger than 1 cm².

4. Praziquantel 40 mgr/kg of body weight was given to the infected population and people was observed for at least 4 h afterwards.

5. Post-treatment evaluations at 3, 6, 9 and 12 months, including coprologic and serologic examinations, were performed.

6. All serologic evaluations were based on both the Circumoval Precipitating Test (COPT) and the Enzyme Linked Immunosorbent Assay (ELISA) with soluble egg antigen of *S. mansoni* (SEA) and adult worm antigen (AVA).

COPT was performed based on the original technique described by Oliver-Gonzalez (1954). Once the infected livers are collected, they are digested in hypertonic solution (1.85%) with trypsina 0.5 mg/ml in steady agitation at 37 °C for 1 h. The liver suspension is passed through 4 screens of 420, 177, 144 and 44 µm size pore. Eggs are collected from the smallest size pore screen and the suspension adjusted to 50 egg/10 µl of hypertonic solution.

Mature eggs were considered positive when precipitation, regardless size, number or shape, were observed around the eggs.

The COPT was positive when the proportion between positive and negative eggs was > 15%, weak positive when it was between 10-15%, and negative < 10% (Spencer et al., 1991). We also have the criterium of the reactivity of the COPT, high reactivity when COPT

is > 50%, medium reactivity when COPT is in the range of 25 to 50% and low reactivity between 10 to 25%.

SEA preparation was based on the Boros and Warren technique (Boros & Warren, 1970), with a modification in the centrifugation speed, which was 9148 G. Adult worm antigen (AWA) was prepared following the technique described by Colley et al. (1977), with the centrifugation speed of 9148 G. For the immunoenzymatic assay, we followed the technique described by Voller et al. (1976). Dynatech 2 plates, anti-IgG alkaline phosphatase system, serum dilution of 1/200 and volumes of 100 µl, were some technical features of the standarization.

RESULTS

The prevalence in the area resulted in 10.37%. One hundred sixty five persons showed eggs of *S. mansoni* in the stools. The average egg excretion diminished progressively with age. Majority of cases (88.4%) showed less than 100 eggs/g of feces, 7.8% had moderate infection and only 3.6% presented severe infection as they eliminated more than 300 eggs/g of feces (Table I). Five out of the six persons with severe infection were less than 15 years old.

Fecal stool examination was not a good criteria for cure, since it was negative in all persons after treatment whether or not they had positive COPT.

Intradermal test results are shown in Table II. The general sensitivity was 58.2%, being less sensitive in women than in men (37.7% vs 62.5%). The sensitivity increased with age, being low in children as 43% and higher in persons older than 30 years (88% sensitivity).

General sensitivity of the COPT test was 95.6% (Table III).

Fifty two individuals with less than 23 eggs/gr of feces, were correctly detected by COPT in 92% of the cases. With the exception of children under 5 years old who showed a COPT sensitivity of 86%, all other age groups have sensitivities higher than 90% (Table III).

The major proportion of medium and high serum reactivity in COPT was observed in the age groups from 6 to 15 years old (Fig. 1). Sex and intensity of infection did not show correlation with COPT results (two ways ANOVA).

TABLE I

Distribution of *Schistosoma mansoni* intensity of infection according to age

Age group	Average group (eggs/g of feces) $\bar{x} \pm s$	Intensity of infection (eggs/g of feces)		
		0-100	101-300	300
0 - 5	154 ± 379	6	0	1
6 - 10	101 ± 256	35	6	3
11 - 15	46 ± 72	43	7	1
16 - 20	23 ± 28	15	0	0
21 - 30	30 ± 34	23	0	0
31 - 40	41 ± 79	16	0	1
40 & +	23 ± 27	8	0	0
Total		146(88.4%)	13(7.8%)	6(3.6%)

 $\bar{x} \pm s$ - mean ± standard deviation.

TABLE II

Distribution of *Schistosoma mansoni* infected patients according to intradermal test result and age

Age group	Area (mm ²) $\bar{x} \pm s$	<i>S. mansoni</i> infected people	Positive Persons	Sensitivity (%)
0 - 5	82 ± 56	7	3	42.8
6 - 10	103 ± 68	44	21	47.7
11 - 15	112 ± 83	51	23	45.0
16 - 20	201 ± 184	15	11	73.3
21 - 30	140 ± 89	23	16	69.5
31 - 40	210 ± 155	17	15	88.0
40 & +	197 ± 113	8	7	87.5
Total		165	96	58.2

 $\bar{x} \pm s$ = mean ± standard deviation.

TABLE III

Circumoval precipitin test results distributed by age in the *Schistosoma mansoni* infected persons

Age group	COPT			Cases	Sensitivity COPT > 10%
	Negative < 10%	Weak 10-15%	Strong > 15%		
0 - 5	1	0	6	7	85.7
6 - 10	1	2	33	36	97.2
11 - 15	2	1	40	43	95.3
16 - 20	0	2	10	12	100.0
21 - 30	1	1	20	22	95.4
31 - 40	1	1	11	13	92.3
40 & +	0	0	5	5	100.0
Total	6	7	125	138	95.6

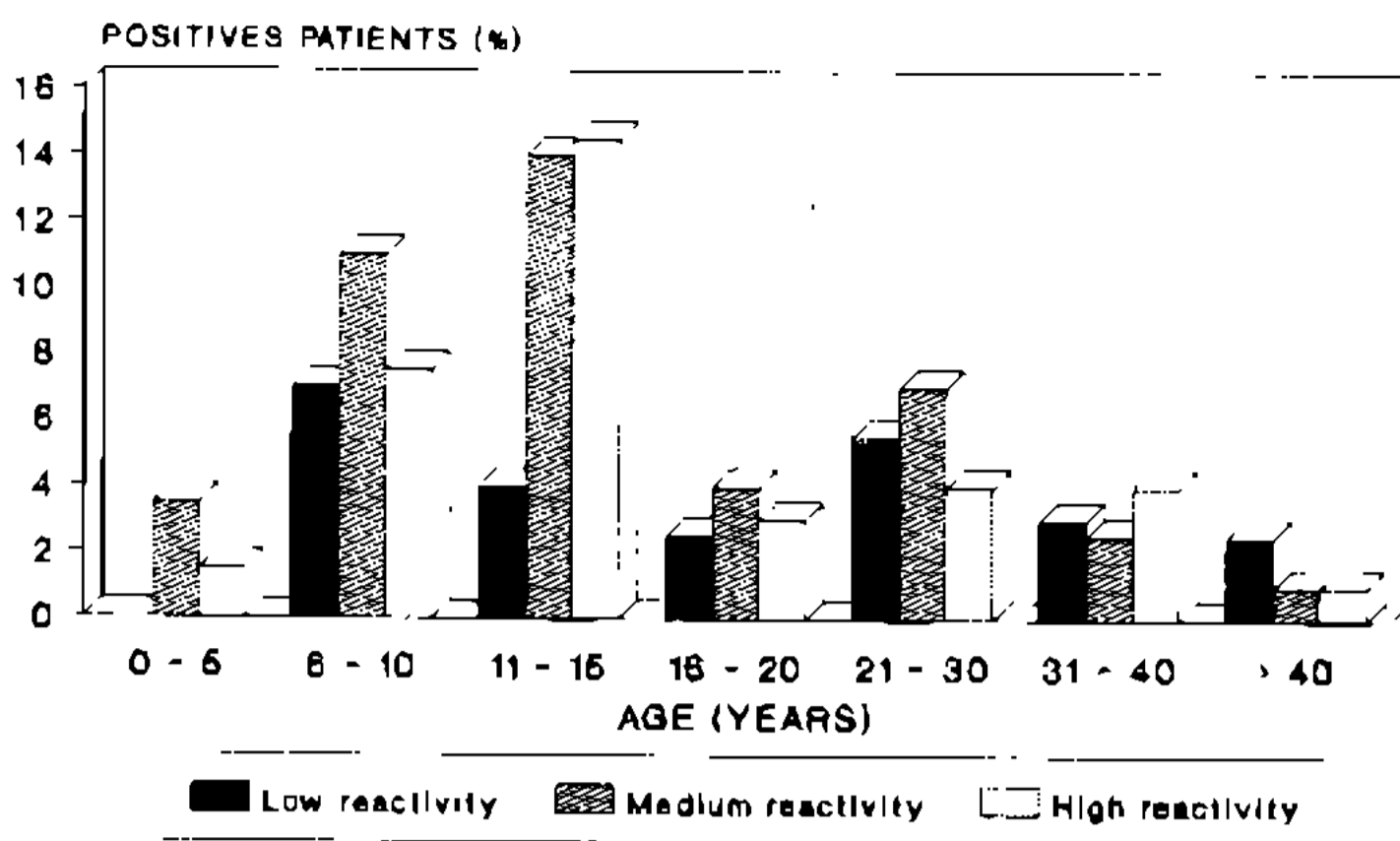


Fig. 1: intensity of the circumoval precipitin test reactivity according to age in *Schistosoma mansoni* infected patients.

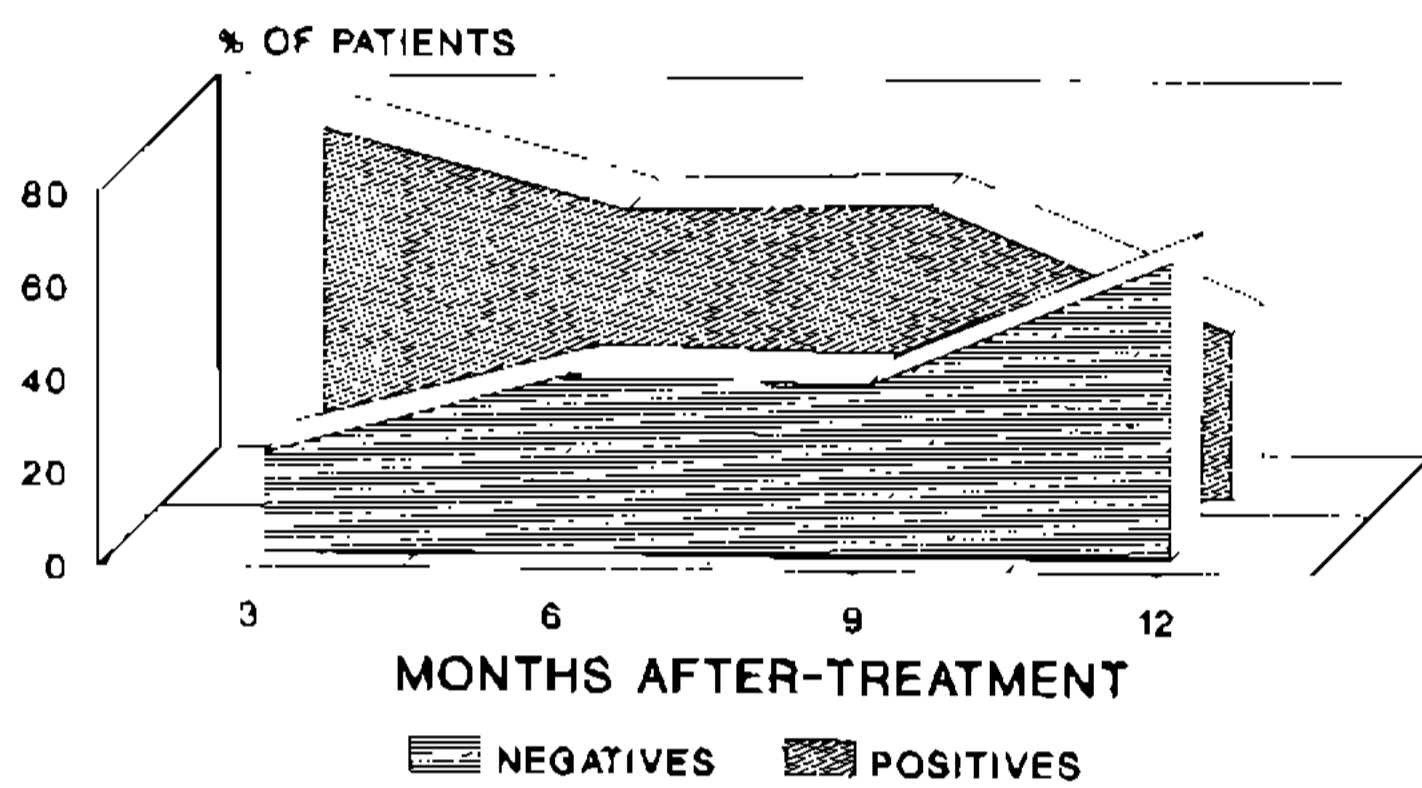


Fig. 2: circumoval precipitin test evolution in praziquantel treated schistosomiasis patients.

Results of post-treatment serologic tests dynamic are shown in Table II.

Not all the infected groups showed an adequate post-treatment follow-up. The percentages of people who complied with the study were 31% at 3 months, 47.8% at 6 months, 18% at 9 months, and 35% at 12 months.

From the observations in each period, 21% of the people seen 3 months after infection became negative to the COPT, 38.5% became negative at 6 months, 37% at 9 months and 63.8% at 12 months (Fig. 2). Cases were valid for the study until they showed negative results.

Those persons who followed adequately the study were classified according to their COPT reactivity. When the treatment is effective, the COPT reactivity progressively diminish until reaching less than 10% one year later. In the non cured group, the COPT reactivity diminish but never below 20% of reactivity.

The 94% of the non-cured patients (33/35) showed medium and high reactivity to COPT before treatment. Of note 67% of this group were children under 15 years old (22/33).

Sensitivity of the ELISA in these 165 persons with SEA an AWA 86%. There was not significant difference among the optical densities in ELISA before and one year after treatment.

DISCUSSION

Forty five years of antibilharzian campaign has not achieved eradication of the disease in our country. Probably the weakest point has been a strategy directed almost exclusively to the vector elimination (Incani, 1987).

The availability of new drugs of low toxicity, easy administration and high effectivity has led the new strategy directed against the parasite (WHO, 1985).

To control the disease with mass chemotherapy, precise diagnostic test must be available in order to have an adequate prevalence indicator.

In countries with high egg elimination rate (> 300 eggs/g of feces) as Brazil, the mass diagnosis based on stool examination is reliable and the prevalence of the disease in school age children is utilized as the parameter for mass chemotherapy (WHO, 1985).

In Venezuela the transmission features seem to be different. In the present work, the majority of the persons (88%) showed low parasitic load with less than 100 eggs/g of feces. From this group, 41% of cases were not detectable by Kato-Katz. One procedure of Kato-Katz per person do not cover those individuals with less than 23 eggs/g of feces which is the inferior limit of sensitivity for the technique.

Then, it is clear to us that the immunodiagnostic tool is the only strategy to be followed in order to assess the impact of Schistosomiasis in our country. The requirements for the optimal immodiagnosis of schistosomiasis have already been established by Mott & Dixon (1982). As schistosomiasis is a chronic disease, that does not need a rapid result, samples can be transported and processed in an adequate laboratory. This fact allows the evaluation of larger number of samples under better conditions.

Eventually, this is not the situation for some African countries where the schistosomiasis foci are far from the cities and they need immunodiagnosis applied to field conditions.

We evaluated three tests in the present work. The intradermal test because it is practical and economic; the COPT for its high sensitivity and specificity, and the ELISA for its application to mass diagnosis. We evaluated the dynamic of seroconversion of the COPT and ELISA after treatment.

The ID test results of venezuelan patients are essentially the same as those reported elsewhere (Alarcón et al., 1987; Hiatt et al., 1978). ID test had low sensitivity in children and women. Some children, even with high egg elimination, resulted negative to it. ID test can not be utilized in mass diagnosis since false negatives rate is high. Hiatt et al. (1978) call the attention about the limitations of the test. However its specificity might help to evaluate the contact of certain population to the parasite.

Besides its high sensitivity and specificity, COPT has the advantage of becoming negative after treatment, which has been demonstrated both experimentally (Cancio et al., 1967) and in humans (Rifaat et al., 1969).

Rifaat et al. (1969) studying 193 *S. mansoni* infected person found that 98.8% of them became negative 10 months after treatment. In the present study only 62% of infected people treated with praziquantel became negative to the COPT one year later. From the public health point of view, this fact does not represent a transmission risk, since eggs disappear completely from the stools. However, it is possible that some worms remain in the liver laying eggs when the cure is not complete. There are evidences that parasites might remain alive after treatment since serologic changes are seen in patients after several treatments (Da Silva et al., 1976). The evolution of COPT reactivity of the non-cured patients indicate that it is not worthwhile to wait a year, in order to conclude whether a patient will become cured. It seems that when the patients cure, the reactivity start to diminish three months after treatment, and by six months, the serology is negative.

Patients under 15 years showed the highest reactivity to COPT.

They also were the predominant group in those who did not cured at all. Butterworth & Hagan (1982) have pointed out that during early infections in children, a strong antigenic stimu-

lus is produced by parasite eggs which is composed by polisacharides or glicoproteins. This antigenic stimulus is the responsible for the high levels of IgM and IgG in serum, which can behave as blocking antibodies probably interfering the antibody-dependant praziquantel action. Attention must be directed to this age group, responsible for the maintenance of transmission.

The use of the COPT is limited due to the need of maintaining the life cycle of the parasite in the laboratory. It is a time consuming technique, and there is not uniformity in the interpretation of the test. Nevertheless, as it is the only test that discriminate parasitic activity, an effort should be made to isolate those fractions of *S. mansoni* eggs which can evaluate effectiveness of the treatment.

ELISA was not useful for the purpose of identifying cured patients. Its application must be reserve for mass diagnostic surveys.

Antigen detecting techniques are been studied for evaluation of effectiveness of chemotherapy (De Jonge et al., 1989, 1991). They indicate that a considerable group of people do not respond adequately to the treatment with praziquantel, although the levels of circulating antigens decrease. Detection of circulating antigens in people excreting few *S. mansoni* eggs is not very reliable yet, therefore studies of impact of chemotherapy in areas like ours with this methodology must be carried out.

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