

Diagnosing Schistosomiasis

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The ideal diagnostic method for schistosomiasis detection seems to be still far from available. Paucity of egg output in low prevalence situations, low levels of circulating antigens in individuals with low intensity of infection and inadequate specificity of antibody detection systems outline pieces of a puzzle that challenges scientific efforts. Estimated prevalence, financial resources and operational reality must be taken into account when deciding the diagnostic method to be used. A combination of a screening step, using a fast strip test for antibody detection with a parasitological ratification step such as Kato-Katz repeated stool examination may serve as a diagnostic approach for a previously untreated low level endemic area. However, when eradication is the aim, and high financial investment is available, re-treatment may be based on the association between multiple stool examination and circulating antigen detection. Ethical aspects as well as cost-benefit rates between treatment and diagnosis approaches lead to the conclusion that in spite of the recent advances in simple administered and relatively safe drugs, treatment should only be performed when supported by appropriated diagnosis.

Key words: schistosomiasis - diagnosis - stool examination - immunodiagnosis

In 1852, Theodor Bilharz, a German physician working in Egypt, described for the first time the parasitic disease which would be after called schistosomiasis. He also presented the first contribution to the diagnostic techniques of the infection: the drawing of the spiculate eggs. These drawings, even containing a conceptual inaccuracy, were the theoretical start for the description of this parasitosis in other regions of the world. Bilharz was mistaken when considering the eggs with terminal spicules and the ones with lateral spicules as pertaining to the same species. This concept was questioned by Patrick Manson (1902) who defined the existence of different species and was defended by Looss (1909) who explained the formation of eggs with lateral spicules as secondary to the excess of vitelline cells in the ootype. The controversy persisted for half a decade until 1907 when Sambon established the new species: *Schistosoma mansoni*. A year later, Pirajá da Silva (1908) described schistosomiasis mansoni in Bahia, Brazil, reinforcing the existence of a different species which caused an intestinal aggression in the infected patients and whose eggs presented a lateral spicule.

In 1919, Adolf Lutz described the first modification in the diagnostic method of schistosomiasis

through the homogenization and sedimentation of feces.

In the beginning of this century scientific advances in immunology occurred. The reaction of complement fixation was developed by Bordet and Gengou in 1901, and the concept of antibodies was established gradually (they were called "amboceptors"). The method of complement fixation was then applied for abdominal typhoid fever and for syphilis by Wasserman et al. in 1906.

In 1909 Fujinami and Nakamura described the reaction of complement fixation for the diagnosis of schistosomiasis. The same method was used by Fairley (1919) with antigens of hepatopancreas of infected mollusks. Since then the use of diagnosis techniques for schistosomiasis has followed the medicinal-laboratorial technological development.

A curious example of consequences of the technological development refers to the history of schistosomiasis. Until few years ago, the observation of the most ancient existence of the infection belonged to Ruffer (1910) who related the presence of eggs calcified in the kidneys of two Egyptian mummies of the 20th dynasty (1250-1000 BC). With the detection of circulating antigens of the parasite in the tissues of the Egyptian mummies, we now know that the humanity lives together with schistosomiasis since 3,000 years BC (Miller et al. 1992).

The study and development of new diagnostic techniques for schistosomiasis are still necessary to investment in view of the reality of the efforts to control the disease. After the 70s, with the coming

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of drugs used in large scale, the specific therapeutic started to play a crucial role in all programs for controlling the disease. The cure of 60 to 90% of the infected people and the reduction 90 to 95% in the number of eggs eliminated in feces are obtained by the treatment with a single dose and few collateral effects. The effective and relatively safe treatment of the infection cooperated with the prioritization of the morbidity control instead of interrupting the transmission in most of the countries where the disease is endemic.

At present, we have three main schemes for the population treatment in control programs of schistosomiasis: (a) *mass treatment*, which proposes a medicament treatment of a whole population possibly infected, without an individual diagnosis; (b) *selective treatment of population*, for groups probably infected or in risk of infection, in which population groups are treated; for instance, the treatment of individuals in the age group from 10 to 20 years of age, who are susceptible to the hepatosplenic form or groups of individuals who perform risk activities for the locality; (c) *selective treatment of individuals*, when only the patients proven to be infected are treated.

The main premise in favour of the mass treatment are the economy of financial resources, the time spent in the stage of diagnosing the infection and the simplification of technical workability of the program. According to the World Health Organization (WHO 1993), epidemiological data indicating a high prevalence of infection in the beginning of the program may justify the treatment of the whole population, without an individual diagnosis.

The global estimate cost of the treatment varies from US\$ 1.5 to 6.5/person (the calculation does not include operational costs: people training, transportation and conservation of the drug, relief for collateral effects). The estimate cost of the feces examination by the Kato-Katz method is US\$ 0.3/person (excluding the operational costs: people training, collecting bottles, microscopes). In several countries where schistosomiasis is an endemic disease, the governmental outlay with public health per year per person is 1.0 to 4.0 US\$ (WHO 1993).

As it will be shown, in an area of 50% of prevalence, the sensitivity of the examination of a feces sample is 90%. Thus, an unnecessary treatment of 50% of the population is carried out with an average cost 10 times higher than what was invested in the diagnosis. Thus, the mass treatment of a 1,000 population with a 50% prevalence entangles in costs of US\$ 3,000 and the unnecessary treatment of 500 persons. The stage of diagnosing the population results in an outlay of US\$ 300 and the correct diagnosis of 450 in 500 infected persons. The

cost becomes US\$ 1,350 for the treatment and more than US\$ 300 for the diagnosis, that is US\$1,650. The problem with this strategy is the 50 false negative patients consequently not treated. These are patients with a low shedding of eggs in their feces, with less chance of developing serious forms and which may be reevaluated in the following stage in a well established longitudinal program. The resource which was saved with the unnecessary treatment, makes possible the repetition of the feces examination, highly increasing its sensitivity (Rabello et al. 1992).

In parasitosis control programs, the decision of the strategy of mass treatment or selective treatment of the population must consider the disadvantages and the risks of not treating patients with a light infection or an unnecessary treatment of not infected individuals including the availability of medical assistance to undesirable serious effects of the medicines. All of the drugs available for the specific therapeutics of schistosomiasis present side effects. The praziquantel is considered as "exceptionally well tolerated" (WHO 1993, Cioli et al. 1995). The reactions described are abdominal discomfort (50-62.5%), bitter taste (62.5%), dizziness (37.5%), sleepiness (25%), asthenia (50%), nausea (5-12.5%), vomit (6.7%), diarrhea (13%), chronic headache (5%), indisposition (2.5%), urticarial reaction (1.7%) (Katz et al. 1982, 1983). With oxamniquine, dizziness (40%), fever (38%), abdominal pain (20%) and chronic headache (21.7%) are observed. The most serious collateral reactions affect the central nervous system. Convulsions of the great harm type, electroencephalographic abnormalities (20%) and visual or auditory hallucinations (0.4 to 0.8%) are also reported (Katz et al. 1983, Foster 1987).

The method of diagnosis consists in a primordial instrument for the programs of control of this endemic disease. It is highly desirable the development of techniques still simpler and more sensitive than the feces examination.

SCHISTOSOMIASIS' DIAGNOSIS METHODS

Until around ten years ago, the diagnosis for certainty of infection by *S. mansoni* could be reached only through the direct demonstration of the parasite in one of its evolution forms in the tissues or excretions of the host. As a result of technological efforts and advances which occurred in the last decade, the detection of antigenic components of the parasite shed in the blood current of the host, became an alternative for the direct diagnosis of the active infection.

Nevertheless, the microscopic demonstration of the parasite's eggs in the feces remains as the more largely tool used, mainly because of its low opera-

tional cost and its feasibility in situations of precarious laboratorial structure. Although some of the techniques of the qualitative diagnosis present a good sensitivity detecting the infection by *S. mansoni*, quantitative techniques are recommended. The determination of the average and of the distribution of the number of eggs per gram of feces reflects the intensity of the schistosomal infection in a population and it makes possible to evaluate the useful indicators in the control planning, such as possible risk factors, presence of severe clinical forms, degree of transmission in the area, expected percentuals of cure and reinfection and intervals of necessary retreatments. Based exclusively in determining the prevalence, the evaluation of the results reached with the control measurements introduced in an endemic region may fail. It is fundamental to observe the repercussions which occurred in the intensity of the infection in the treated population.

PARASITOLOGICAL DIAGNOSIS

Regarding the available techniques for the parasitological feces examination, in the most recent publications it remains the consonance that the Kato-Katz method (Katz et al. 1972) offers the best conditions of effectiveness associated to the cost and operational conditions (Mott & Cline 1980, Sleight et al. 1982, Rabello et al. 1992, WHO 1993).

A significant increase in the sensitivity of the parasitological feces examination by the Kato-Katz method may be reached by the exam of a larger number of fecal samples. This procedure is mainly indicated when the population group studied presents a low prevalence and a low intensity of infection and in the control of cure, situations in which the expected number of eggs in the feces is small. The sensitivity of the examination of only one fecal sample compared to the examination of four samples was of 84.9% and 100% when the number of eggs per gram of feces was greater than 50, 100 and 500, respectively, in an endemic area of low prevalence and intensity of infection (Rabello et al. 1992).

Alternatives such as the use of mathematical models to estimate the population prevalence may help in the control planning. Through the development of a mathematical model based on the individual variations of parasitic burden, variations in the number of worm pairs for a certain parasitic charge and in the variation in counting the eggs for a certain number of worm pairs, De Vlas et al. (1992a) established a prevalence curve estimated for feces exams with multiple samples from the prevalence observed with a single feces exam. This model was validated by De Vlas et al. (1992b) by using data of Jordan et al. (1975) with prevalences

of one and three feces examinations in eight localities of Santa Lúcia and by De Vlas et al. (1997) from observations of seven different population groups.

When the choice of the schistosomiasis diagnostic method aims to evaluate individual patients, the demonstration of the parasite's eggs in fragments of rectal mucosa is sometimes considered. Regarding this technique it is worth emphasizing: (a) if the criteria of positivity is standardized as the presence of viable eggs in the rectal tissue, and the technical quality of the examiner of the fecal samples is guaranteed and of the oogram, two feces examinations by the Kato-Katz method present the same sensitivity that the oogram obtained by the rectal biopsy; (b) due to the significant and positive correlation between the number of eggs observed in the rectal tissue and in the feces, the concordance between the two methods is 100% when the patient presents more than 200 eggs per gram of feces and more than 2,000 eggs per gram of rectal mucosa. The discordances are observed in the infections which are less intense (Rabello 1992); (c) at present, the indication of the rectal biopsy for the diagnosis of schistosomiasis mansoni is restricted to the drugs assays, when the early demonstration of modifications in the rectal oogram helps in the evaluation of the therapeutic effectiveness.

IMMUNOLOGICAL DIAGNOSIS

Several groups of researchers have been looking for developing methods of immunological diagnosis which present a high specificity and sensitivity and which constitute alternatives for the use of parasitological techniques. The techniques available are numerous but up to the moment none of them satisfy completely the requirements for an "ideal technique" which besides being effective must use a unique reagent, depend on simple equipment, be fast, be of low cost and easy to be done in the field. The skin-test, based on the immediate hypersensitivity reaction, as a simple technique, is the one which is closer to be the "ideal" one, but as it presents a low specificity for the infection in activity (Rabello 1990), it was discharged.

The collection of material in filter paper or in microtubes, or the possibility of using total blood reduce the difficulties which constitute the venous collection, the blood centrifugation in field and its transport refrigerated for the laboratory. A recent advance, promising for the use of simplified procedures was reported by Garcia et al. (1995), describing the detection of antibodies IgG against antigens of *S. mansoni* eggs in saliva and in the oral transudate of infected patients, with similar sensitivity and specificity as those obtained with serum.

DIAGNOSIS FOR ANTIBODIES DETERMINATION

The first and indispensable attempt to standardize and evaluate several assays based in the determination of specific antibodies was performed by Mott and Dixon in 1982. In a multicentric study, the authors evaluated 17 antigenic preparations and tests in a total of 248 sera of infected patients and 88 controls of uninfected patients. The tests of indirect hemoagglutination, indirect immunofluorescence, ELISA, periovular, radioimmunoassays, indio-immunoassay and double microdiffusion were effectuated with different antigens. The authors conclude that the results did not indicate superiority of none of the immunodiagnostic methods for detecting anti-schistosoma antibodies.

The researches in this field have concentrated their attention in search of purified antigenic components which induce the formation of more specific antibodies than the other existing ones. Purified preparations as CEF6 (cationic fraction 6), MSA1 (major serological antigen), MAMA (adult microsomal antigen), 37 Kda (37 larval antigen and 31/32 gut associated) have been described as possible alternatives, but they still did not prove their usefulness for the use in large scale as substitutes for parasitological methods (Bergquist 1992, 1993, Feldmeier & Poggensee 1993).

The CEF6 antigen constitutes an example of the necessity of validation in areas of apparently specific antigens. When this antigenic fraction was evaluated by Mott and Dixon (1982) it was considered a promising one. The specificity of 93.3% was observed in 33 Europeans and Amazon indians and of 80% in 17 patients treated for schistosomiasis mansoni. While studying the diagnosis with the same antigen in Kenya, Doenhoff et al. (1993) observed a specificity of 100% with SEA and of 98% with CEF6 in 254 children of a non-endemic area. Unfortunately, however, specificity of 64% and 59% were observed with SEA and CEF6 respectively, in 887 individuals who live in an endemic area for *S. mansoni* and less than 20% for both antigens in 234 children who live in an endemic region for *S. mansoni* and *S. haematobium*. In this case, the purification of the antigen did not result in a greater specificity. Recent technological advances may contribute to the finding of a specific humoral reply. The facility of producing recombinant antigens and synthetic peptides offers a perspective of finding specific epitopes for the active infection (Klinkert et al. 1991, Lo Verde et al. 1992).

The confrontations of the data of prevalence of one endemic region obtained by feces examination and determination of antibodies generally result in a higher positivity of the serological exami-

nation (Polderman et al. 1985, Shimizu et al. 1992). There are two possible categories: excess of false positives per serological evaluation or excess of false negatives per parasitologic interpretation. The low specificity of the presence of antibodies against *S. mansoni* in the absence of an active infection may be explained by the cross reactivity with other parasites (Corrêa-Oliveira et al. 1988), unisexual infections, contact with other cercariae, transference of maternal antibodies, previous treatment ignored and persistence of antibodies after cure.

Other alternative described is based on the use of enzymes pertaining to the worm in possible immunodiagnostic assays. A sensitivity of 90% was reached by identification of antibodies against the alkaline phosphatase present in the worm's tegument. Until now, from 17 reported enzymes, this was the only which was surveyed in the diagnosis of the infection but it did not offer any advantage on the rough antigens in the differentiation between previous and active infection (Cesari et al. 1992).

Data of the literature suggest that the diagnosis of schistosomiasis based on the determination of specific antibodies against the antigens presently available must be established only in countries where schistosomiasis is not endemic. It can also be used for prevalence estimates in populations not previously treated, but the low specificity and consequent low positive predictive values makes the method inadequate for studies of prevalence and control (Spencer et al. 1991).

DIAGNOSIS PER ANTIGEN DETECTION

Detecting antigenic substances released by the parasite, per definition, constitutes the procedure which makes possible to differentiate a past infection from an active one eliminating the problem of low specificity of the diagnosis of antibodies.

The specificity of 100% is the main advantage that the detection of circulating antigens offers in relation to the antibodies research. On the other hand, in what refers to the sensitivity, the "gold standard" test for schistosomiasis has not been achieved yet. In the populations of Burundi and Zaire, sensitivity of 59.6% and 66.7% were observed in people with less than 100 eggs per gram of feces (De Jonge et al. 1991).

As part of a multicentric study including endemic areas of Minas Gerais, Brazil no significant difference was demonstrated in 116 people of positivity from one (46.6%), two (52.6%) or three (59.5%) feces examinations and the detection of an anodic circulating antigen in the blood (54.3%) and that in discrepant cases are predominantly observed in patients with a low shedding of eggs in their feces and low levels of circulating antigens

(data non published). Similar equivalence was observed by Van Lieshout et al. (1995), in a low prevalence area from Surinam, comparing two fecal samples - 25 mg Kato-Katz examination to CAA and CCA detection in sera and urine. In this study, the highest sensitivities were achieved with the urine-CCA assay and the parasitological examination, detecting 59 and 58 out of the 107 cases with an active infection.

Several circulating antigens have been described in different laboratories and enough research to allow the multicentric evaluation was reached by some of these assays (De Jonge et al. 1988, Barsoum et al. 1991, Qiu et al. 1991, Hassan et al. 1992).

The excretion of circulating antigens of the worm in the urine of the infected patients makes possible the use of a non-invasive technique for the simple diagnosis of schistosomiasis. Sensitivity and specificity are similar to those reported for the serological detection of antigens (Delder et al. 1989, Van Lieshout et al. 1993). Low fluctuations of urinary elimination of these antigens and the number of eggs shed in the feces during seven collections in five days and three subsequent weeks have been observed (Van Etten et al. 1996, Disch et al. 1997).

The disadvantages of the circulating antigens detection are low sensitivity in light infections, high cost, difficult approach and dependence of the production of monoclonal antibodies. This technique, which is still far for being used in control programs, has received investments such as choice technique for future evaluation of protection assays. It also consists of a good method of research, such evaluation of circulating antigens and morbidity relation (De Jonge et al. 1991).

TECHNICAL VALIDITY AND APPLICABILITY

Fig. 1 presents four possible categories of disease classification by a diagnostic method and its relation with the truth situation and its interrelations.

Sensitivity is defined as the capacity of a certain technique of detecting the greatest number of individuals truly sick. Specificity is the capacity of the test being always negative in the absence of the disease, not offering false-positive results. Effectiveness is the propriety of correctly classifying the greatest number of evaluated individuals, sick and healthy ones.

The interrelations above do not depend exclusively on the test's characteristics. The predictable values incorporate in their definition an aspect of prevalence of the disease in certain population. Maintaining the characteristics of sensitivity and specificity of the test, the probability of an indi-

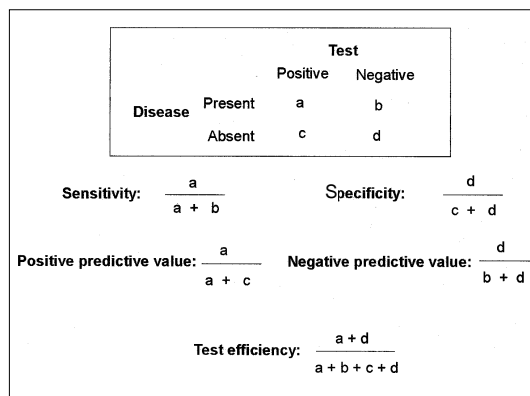


Fig. 1: four possible categories of disease classification by a diagnostic method and its relation with the real situation and its interrelations.

vidual who presents a positive test to be really sick (predictable value of the positive result) depends on the specificity of the test and on the prevalence of the disease in the population to which the individual belongs. The probability of an individual who presents a negative test to be truly healthy depends on the sensitivity of the test and on the prevalence of the disease in the population.

The example presented in Fig. 2 shows how the predictive values depend on the situation where a certain diagnostic test is performed.

The feces examination for the diagnosis of parasitosis such as schistosomiasis presents two aspects which need to be considered when evaluating its sensitivity and predictive values. First, unless by changing results (virtual mistake), the test specificity is 100%. When the egg of *S. mansoni* is visualized, there is no chance of being a false positive patient. Second, as mentioned above, the sensitivity of the method increases with the growth of the infection intensity.

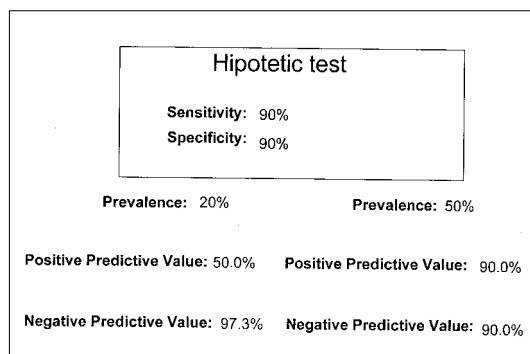


Fig. 2: predictive values of a hypothetical diagnostic test.

Fig. 3 presents the linear relation between prevalence and infection intensity of the experience in 10 endemic areas in Minas Gerais, evaluated by the Laboratório de Esquistossomose of the Centro de Pesquisas René Rachou.

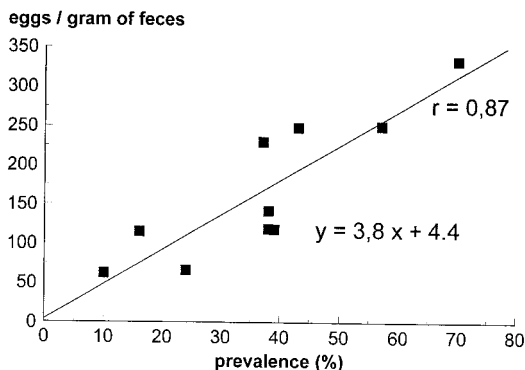


Fig. 3: linear regression between prevalence and geometric mean of the number of eggs per feces gram obtained in 10 endemic areas evaluated by the Laboratório de Esquistossomose, Centro de Pesquisas René Rachou, FIOCRUZ.

Thus, it is expected to find an approximate geometric mean of 70 eggs per gram of feces and sensitivity of the examination of a fecal sample of around 70% for an endemic area with a 20% prevalence. For an endemic area of 50% it is expected an approximate geometric mean of the number of eggs of 200 eggs per gram of feces and sensitivity of 90%. The consequences of the variation can be seen in Fig. 4, in hypothetical data.

Notwithstanding the great number of tests applicable to the serological diagnosis, described as presenting high sensitivity and specificity in initial studies, few have resisted to more careful ob-

Kato-Katz Method Diagnosis of Schistosomiasis	
Prevalence: 20%	Prevalence: 50%
Intensity of infection: 70 opg	Intensity of infection: 200 opg
Sensitivity: 80%	Sensitivity: 90%
Specificity: 100%	Specificity: 100%
Positive Predictive Value: 100%	Positive Predictive Value: 100%
Negative Predictive Value: 95.2%	Negative Predictive Value: 90.9%

Fig. 4: epidemiological indicators of the Kato-Katz method in two hypothetical localities.

servations in terms of effectiveness, reproducibility, cross reactivity and positive and negative predictive values, which are indicators that depend on the prevalence in the area studied. It is still expected that according to the obvious social composition and geographical situation of the population affected by schistosomiasis to use the appropriate methods and technology and not the opposite.

In summary, the technological development and the several advances reached by immunodiagnostic tests present promising results. However, we are still in search of the ideal test which surpasses the development stages in laboratory, minimum criteria of validation and applicability in the field. This test needs to be superior to the feces examination of a single sample.

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