

THE VALUE OF AN IMMUNOENZYMATIC TEST (ENZYME-LINKED IMMUNOSORBENT ASSAY) FOR THE DIAGNOSIS OF STRONGYLOIDIASIS IN PATIENTS IMMUNOSUPPRESSED BY HEMATOLOGIC MALIGNANCIES

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Abstract. The diagnosis of strongyloidiasis relies upon the identification of the parasite in stool samples. In 1981, a serologic assay was developed, which was useful in the diagnosis of strongyloidiasis in the immunocompetent host. In the present study, we evaluated the enzyme-linked immunosorbent assay (ELISA) in patients with hematologic malignancies. Between April 1995 and December 1998, sera from 164 consecutive patients were tested for the presence of IgG antibody to *Strongyloides stercoralis*. Patient was considered uninfected after at least three negative stool examinations. The prevalence of strongyloidiasis was 13%. The underlying diseases were acute leukemia in 21% and lymphoma in 52% of the patients. The majority of the patients were receiving chemotherapy (93%) and steroids (76%). The sensitivity, specificity, and positive and negative predictive values were 68%, 89%, 48%, and 95%, respectively. The ELISA may be an excellent assay to rule out the diagnosis of strongyloidiasis in patients with hematologic malignancies.

INTRODUCTION

Strongyloides stercoralis is an intestinal nematode with worldwide distribution, but especially common in tropical and subtropical areas. The clinical course of strongyloidiasis is usually benign, with few symptoms such as mild diarrhea. However, there are some case reports of an invasive form of the disease characterized by septic shock and meningitis following an increase in the parasite load. This form of strongyloidiasis, called disseminated syndrome, is associated with high mortality.^{1–3} Immunosuppression is a risk factor for severe strongyloidiasis and patients with hematologic malignancies are especially at risk because they are immunosuppressed due to their underlying disease, as well as to its treatment.^{4–7} Indeed, leukemia and lymphoma account for up to 90% of the cases of malignancy-associated severe strongyloidiasis.⁸

The diagnosis of strongyloidiasis relies on the identification of the parasite in stool samples or, rarely, in sputum and biopsies. The Baermann-Moraes method and fecal culture in agar are the most sensitive and specific methods used to detect the larvae of the parasite.^{9,10} Unfortunately, false-negative results are frequent, the main causes being the variable amount of larvae in stool collected at different periods, the necessity to analyze at least three stool samples, and the difficulty in performing the method.^{11,12}

The knowledge that specific antibodies against antigens of the filarioid larvae of *S. stercoralis* are synthesized by the immune system after the infection led to the development of an immunodiagnostic test to detect IgG specific for the parasite. Both an indirect immunofluorescent assay and an enzyme-linked immunosorbent assay (ELISA) were tested in immunocompetent patients, and the sensitivity and specificity ranged from 70% to 90% and 85% to 95%, respectively.^{13–18}

Immunocompromised patients may have an altered immune response to *S. stercoralis*. This issue has been analyzed in two studies with contrasting results. The sensitivity of the ELISA in immunosuppressed patients was only 10% in one study and 80% in another.^{19,20}

In this study, we prospectively evaluated the sensitivity, specificity, and predictive values of the serology for *S. stercoralis* in a population immunosuppressed by hematologic malignancies.

PATIENTS, MATERIALS, AND METHODS

The University Hospital, Universidade Federal do Rio de Janeiro, is a public teaching hospital that receives mainly patients with poor economic status coming from surrounding urban areas. The study protocol was approved by the Institutional Review Board of our institution. All patients admitted to the Hematology Service between April 1995 and December 1998 who satisfied the eligibility criteria were asked to participate in the study after signing an informed consent form (consent was obtained from the parents or legal guardians in the case of minors). The eligibility criteria were the presence of a hematologic malignancy (acute or chronic leukemia, lymphoma, multiple myeloma, or myeloproliferative disease) and appropriate stool and serum collection.

Each stool sample was analyzed by direct examination and the Hoffman and Baermann-Moraes methods. A patient was considered not infected if at least three stool samples collected on different days were negative for the presence of *S. stercoralis* by each of the three methods. Patients with less than three stool samples were excluded from the analysis unless they were found to have strongyloidiasis. Patients with strongyloidiasis were treated with thiabendazole (25 mg/kg, maximum = 1,500 mg) two times a day for three days.

For each patient, 10 ml of serum was collected during the period of stool examination, and frozen -75°C . Patients whose serum was not collected or those whose date of blood collection exceeded a month from the date of stool collection were excluded from the analysis.

The antigen used was kindly provided by Dr. Franklin A. Neva (Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD). It was obtained from filariform larvae of *S. stercoralis* as reported previously.¹⁷ Briefly, poly-

styrene, round-bottomed, microtiter plates with 96 wells were used as the surface for antigen sensitization. Antigen was diluted in carbonate buffer (pH 9.6) to a concentration of 5 µg/ml and 100 µl of this solution were incubated in the wells for 18 hr at 4°C. The plates were washed five times with phosphate-buffered saline (PBS)–Tween 20 solution, then with distilled water, and frozen at –20°C after drying. All sera were tested simultaneously to avoid using different antigen preparations. Fifty microliters of PBS–Tween 20 was put in each vial of the antigen-sensitized plate. An aliquot of 10 µl of serum diluted in 160 µl of PBS–Tween 20 was added to each well. The solution was incubated for 1 hr at 37°C. Each plate was washed five times with PBS–Tween 20 and 100 µl of alkaline phosphatase–conjugated goat anti-human IgG (A-3150; Sigma, St. Louis, MO) previously diluted in Tween 20 to a titer of 1:1,000 was added to each well. The plates were incubated at 37°C for 1 hr and washed five times with PBS–Tween 20. The substrate, *p*-nitrophenylphosphate (N-9389; Sigma), was diluted to a concentration of 1 mg/ml in bicarbonate-carbonate buffer (pH 9.6) plus MgCl₂. One hundred microliters were added in each vial and the optical density values were obtained after 30 minutes at 405 nm. Each serum was tested in duplicate. The final test value was the mean between the two readings unless one value was above the cutoff point and the other was below it. In this case, the tests were repeated and the patient was excluded from the analysis if the results remained unchanged. Both positive and negative control sera were present in each plate. The cutoff value of the ELISA was calculated as the mean plus three standard deviations optical density of an uninfected population. This mean was established using the sera of 40 patients who had five or more stool samples negative for parasites, pyocytes, or blood by the three methods already mentioned. The cutoff value was 0.184.

We chose a positive control group of 62 patients with strongyloidiasis and no hematologic malignancy. They were selected by identifying consecutive positive stool examinations from the parasitology laboratory during the study period.

For calculation of the sample size, we estimated a prevalence of strongyloidiasis of 12%, based on two studies conducted in Brazil.^{21,22} The number of patients required to estimate a 95% confidence interval for the specificity of the test of 88% was 162. The number of control patients selected to perform the cutoff calculation was determined in the same way, except that the estimated prevalence of strongyloidiasis (98%) was taken from the specificity of a ELISA in immunocompetent patients.¹⁵

The data were analyzed using Epi-Info version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA). *P* values were calculated using the chi-square test or Fisher's test for dichotomous variables. For continuous variables, we used the Student's *t*-test or the Wilcoxon test. The odds ratio and the 95% confidence intervals for the differences between proportions were also calculated.

RESULTS

Three hundred twenty-two patients had at least one stool examined between April 1995 and December 1998. Larvae

TABLE 1
Main characteristics of the patients

Characteristics	Strongyloidiasis	
	Yes (n = 22)	No (n = 142)
Gender: male:female*	18:4	69:73
Age, years (%)		
<20	2 (9)	20 (14)
20–60	15 (68)	79 (56)
>60	5 (23)	43 (30)
Steroid treatment (%)	17 (77)	108 (76)
Antineoplastic chemotherapy (%)	21 (96)	132 (93)
Mortality (%)†	8 (40)	48 (35)
Hematologic malignancy (%)		
Acute leukemia	6 (27)	28 (20)
Lymphoma	8 (36)	77 (54)
Multiple myeloma	2 (9)	19 (13)
Chronic lymphocytic leukemia	2 (9)	10 (7)
Myeloproliferative disease‡	5 (18)	7 (5)

* *P* = 0.008 for the comparison between groups.

† *P* = 0.01 for the comparison between groups.

‡ Six patients without strongyloidiasis and two patients with strongyloidiasis were excluded from the analysis because they were lost to follow-up.

of *S. stercoralis* were found in 12 patients (4%). A second stool test disclosed the parasite in other five cases. Only 199 patients (62%) provided three stool samples and 35 of those had to be excluded from the study (34 had irregularities in the sera collection and one refused to participate in the study). These exclusions affected 19% of the patients with strongyloidiasis and 17% of the patients without strongyloidiasis. One hundred sixty-four patients were analyzed, and 22 were infected by *S. stercoralis* (13%).

A diagnosis of strongyloidiasis was made in the first stool examined in 12 cases (55%), in the second stool sample in five cases (23%), in the third sample in one case (4%), in the fourth in two cases (10%), in the fifth in one case (4%), and in the sixth in one case (4%). Therefore, the positivity of 55% of a single stool tested increased to 82% when three stool samples were analyzed. Other parasites were present in seven cases (32%) with strongyloidiasis and in 41 cases (29%) without this disease. The parasite was *Entamoeba coli* in 18 cases, *Giardia lamblia* in 10 cases, *Ascaris lumbricoides* in six cases, *Trichuris trichiura* in five cases, *Entamoeba histolytica* in five cases, hookworm in three cases, *Enterobius vermicularis* in two cases, and *Iodamoeba butschilli* in one case. None of these parasites were associated with the presence of *S. stercoralis*.

As shown in Table 1, the characteristics of patients with and without strongyloidiasis were similar regarding age, use of steroids, chemotherapeutic regimen, and mortality. The proportion of males was higher in patients with strongyloidiasis (82% versus 49%; *P* = 0.008), as was the proportion of patients with myeloproliferative diseases (18% versus 5%; *P* = 0.01).

The control group was composed of 62 patients with strongyloidiasis and no hematologic malignancy. Fifty-two sera from this sample were positive by the ELISA (sensitivity = 84%). The median optical density ($\pm$ SD) of the control patients was 606 ($\pm$ 39) compared with 425 ($\pm$ 59) for the patients with strongyloidiasis and hematologic malignancy (*P* = 0.01).

Table 2 shows the results of the ELISA. Fifteen of 22

TABLE 2

Results of the serologic test (enzyme-linked immunosorbent assay) for *Strongyloides stercoralis* in patients with hematologic malignancies according to the presence of the parasite in the stool

Serologic test		<i>S. stercoralis</i> in stool	
		Yes	No
Positive	31	15	16
Negative	133	7	126

serum samples from patients infected with *S. stercoralis* had positive results by this method. Therefore, the sensitivity of the immunoenzymatic test was 68%. One hundred twenty-six of 142 serum samples from patients not infected by this helminth showed negative results by the ELISA, giving a specificity of 89%. One patient had conflicting results in the duplicate analysis. The test was repeated and both values were less than the cutoff value, so the sample was considered negative. The positive and negative predictive values of the ELISA in the study population were 48% and 95%, respectively (Table 3).

We analyzed the group of 16 patients with false-positive results. The comparison of characteristics (gender, age, treatment, illness, mortality, stools analyzed and parasites found) of this sample with the 126 patients negative by both the stool and serologic tests (true negative) did not disclose any statistically significant difference. We also analyzed the characteristics (gender, age, illness, treatment, and mortality) of the 22 patients with strongyloidiasis compared with the group with positive serologic test results (true positive) and the group with negative serologic test results (false negative); there was no difference between them.

DISCUSSION

In the present study, the prevalence of strongyloidiasis was very high. However, our result of 13% was smaller than the 21% reported in a retrospective study done at the same institution.²¹ This difference may be explained in part by a selection bias in the previous study, since stool tests were not routinely done in asymptomatic patients with less probability of being infected by the helminth. In a prospective study conducted in patients with hematologic malignancies, the prevalence was 8.3%, a result similar to ours.²² In our study, a selection bias could be present since the mean number of stools examined in infected patients was two, less than the three required by the patient to be considered not infected. Only 64% of the patients had three stool samples examined and some of the other 36% (101 patients) could be undiagnosed. This finding stresses the difficulty in performing repeated stool tests even within a research protocol.

In the comparison between patients with and without strongyloidiasis, male sex was more frequent in infected patients. Male gender is a well-known risk factor for strongyloidiasis. Davidson and others identified five risk factors in a case-control study: black race, male sex, steroids, hematologic malignancy, and gastric surgery.⁴ In the present study, 76% of the patients took steroids and hematologic malignancy was an inclusion criteria for study entry. Race was not evaluated because this variable is very hard to clas-

TABLE 3

Performance of the serologic test for the diagnosis of strongyloidiasis in patients with hematologic malignancies

Characteristics	Value (95% CI)*
Sensitivity	68.0 (45–86)
Specificity	89.0 (83–94)
Predictive values	
Positive	48.0 (30–67)
Negative	95.0 (89–98)

* CI = confidence interval.

sify in Brazil, a place with a high degree of racial mixing. Gastric surgery was not analyzed in our study.

The frequency of different hematologic malignancies reported in our study is not similar to the one found in epidemiologic studies.^{23,24} Chronic lymphocytic leukemia, the most common hematologic malignancy of the western hemisphere, represented less than 10% of our study sample. This may be due to a selection bias because patients with chronic lymphocytic leukemia have fewer consultations and hospitalization than patients with acute leukemia or high-grade lymphomas do; therefore, it is more difficult to have the stool samples collected. Our finding that myeloproliferative disease was more common in infected patients is unexpected and should be confirmed by an epidemiologic study.

The lack of a gold standard for the diagnosis of strongyloidiasis is troublesome.¹¹ The Baermann-Moraes and agar plate culture methods are considered the most sensitive ones.²⁵ However, these tests can give false-negative results because of the irregularity of the oviposition by the adult parthenogenetic female. At least three stool examinations must be done to rule out the infection.²⁶ Dreyer and others followed asymptomatic individuals with strongyloidiasis and performed weekly stool examinations using the Baermann-Moraes method. They found that 66.7% of the patients had positive stool examinations after eight weeks and up to 21 examinations were needed to identify all infected individuals.¹² The mean number of stools tested in our study was five in the uninfected group, varying between three and 13, depending on clinical suspicion. A limitation of our study is that there was no uniformity in the number of stool samples examined per subject. Indeed, 18% of our patients had the diagnosis of strongyloidiasis made only after four or more stool examinations.

Some studies use two standard deviations above the mean as the cutoff limit.^{15,17,19,27} We repeated the calculation using this parameter and it did not change our results.

The sensitivity of the ELISA was lower than the values reported in the literature, which ranged from 80% to 100%.^{14,15,17,28–30} In a previous study done in Brazil in a region endemic for strongyloidiasis, the sensitivity was 92% in the general population of immunocompetent individuals.²⁷ Our lower sensitivity may be explained by the reduction of antibody production because of the immunosuppression. Indeed, the optical density values of the sera from the immunocompromised patients were significantly lower than the values of the control sera. In another study done in immunocompromised patients, the sensitivity was very low. Unfortunately, the description of the population was too vague to compare the degree of immunosuppression between this

study and ours.¹⁹ In another study, 15 immunocompromised patients were compared with more than 100 immunocompetent individuals and there were no differences in the sensitivity of the serologic assay. In that study, the degree of immunosuppression was probably lower than in our study because 66% of the patients had autoimmune disease, and cancer was present in only one case.²⁰

The specificity of the test observed in the present study was similar to the results reported in immunocompetent people, which ranged from 82% to 100%.^{14,15,18,27,30,31} Although sensitivity and specificity are intrinsic properties of any given test, the positive and negative predictive values are the most useful information for the user of the test because they represent the rate of true positive and true negative cases, with the results varying depending on the prevalence of the infection in the study area. In our study, the positive predictive value of the ELISA was very low, reflecting a very high rate of false-positive results (half of the positive results). This finding implies that a positive ELISA result does not correlate well with the presence of *S. stercoralis* in the stools examined by the Baermann-Moraes test. False-positive results could represent a cross-reaction with other parasites, mainly those causing filariasis.^{28,31} However Rio de Janeiro has a low prevalence of this parasite, which makes this explanation highly unlikely. We compared the rates of other stool parasites in false-positive and true-negative patients and did not find any differences. However, this information must be viewed with caution because of the small number of parasites found other than *S. stercoralis*. No other characteristics could differentiate those two groups of patients. The possibility of an occult strongyloidiasis cannot be ruled out mainly because of the limitations of the stool tests.¹¹ The median number of stools examinations performed in the group of false-positive people was four, which is adequate.^{12,26} The persistence of antibodies after the infection was treated could be another cause of false-positive results. Previous studies showed that the level of antibodies decrease after treatment, usually after a period of less than a year.^{30,32}

Many studies propose the hypothesis that the serologic assay might be more sensitive than the stool assay.^{32,33} Bailey studied a group of patients with clinical signs of strongyloidiasis, including larva currens and urticaria, and found high levels of anti-IgG in the absence of larvae in the stools.³³ Grove followed the response to treatment with thiabendazole and described the persistence of high levels of the same antibodies in the patients that had protracted diarrhea after treatment, even with negative stool examination results.³²

The negative predictive value found in our study was very high (95%). However, this data must be interpreted with caution because the prevalence of strongyloidiasis in our study was low. We do not have a good explanation for the false-negative results. This group was not more immunocompromised than the other patients. Misidentification of the rhabditiform larvae of hookworm, because of their similarity to *S. stercoralis*, is a possible explanation. The difference is usually made by microscopic examination of fixed material, a procedure that was not done in the present study. It is possible that patients could be in the acute phase of infection, with no production of specific IgG. This hypothesis is supported by the observation of strongyloidiasis in animals. Genta and others infected a group of dogs with *S. stercoralis*

and analyzed the immune response. The production of IgG was detectable only after the sixth week of infection.³⁴

In conclusion, the immunoenzymatic test seems to be a good screening assay in ruling out strongyloidiasis in patients with hematologic malignancies, in the view of the poor compliance of patients to repeated stool tests. Nevertheless, positive results must be viewed in the context of clinical symptoms because of the high rate of false positive results.

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