Usefulness of *Strongyloides stercoralis* Serology in the Management of Patients with Eosinophilia

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Abstract. *Strongyloides stercoralis* infection is being increasingly diagnosed out of endemic areas. The aim of this study is to evaluate the usefulness of *S. stercoralis* serology for the management of probable strongyloidiasis in patients presenting with eosinophilia. Overall, 147 patients were included, 89 (60.5%) patients had a positive *S. stercoralis* serology. *Strongyloides stercoralis* larvae were detected only in 15 (10.2%) patients. Twenty-eight patients had human immunodeficiency virus infection. Eighty patients received ivermectin 200 mcg/Kg/day for 2 days, and follow-up 6 months after treatment could be performed in 32 patients: 26 (81.3%) patients reached the response to treatment criteria (negative serology 6 months after treatment or when by enzyme-linked immunosorbent assay the optical density ratio of post-treatment to pre-treatment decreased to 0.6), and 11 (34.4%) patients fulfilled the cure criteria (negative serology 6 months after treatment). *Strongyloides stercoralis* serology is a useful diagnostic tool both in the diagnosis of probable strongyloidiasis and follow-up after treatment.

INTRODUCTION

Eosinophilia is a common finding in travelers and migrants, and it often indicates an underlying helminth infection. Eosinophilia was detected in 1,191 of 7,792 (15%) United States-bound migrants attended in two GeoSentinel clinics; from these patients, strongyloidiasis and schistosomiasis were the most frequent diagnosis. Nevertheless, most of the patients presenting with eosinophilia, remained without an etiological diagnosis.¹

An estimated 100 million people are infected worldwide by the intestinal nematode *Strongyloides stercoralis*. This parasite can be found in widespread areas of the tropics and subtropics, but it has been also reported in more temperate climates, like southern Europe and southern parts of North America.² *Strongyloides stercoralis* infection is being increasingly diagnosed in Tropical Medicine Units out of endemic areas not only as a result of migrant movements and ease of traveling, but also because more sensitive tests (serology) are being used for the diagnosis.³

Strongyloidiasis is asymptomatic in most patients, but patients may present with clinical symptoms and signs including cutaneous, gastrointestinal, and respiratory involvement. Eosinophilia is frequently the only finding in patients with strongyloidiasis. This parasite can be permanently established in human hosts without the need of exogenous reinfection because of its autoinfective life cycle. Under some immunosuppressant conditions, this autoinfective cycle could be amplified leading to fatal presentations such as *S. stercoralis* hyperinfection syndrome and disseminated strongyloidiasis.⁴

The confirmatory diagnosis of *S. stercoralis* infection is made on the basis of detection of larvae in the stools. However, in most chronic asymptomatic patients, the intestinal worm load is very low and the output of larvae is minimal and irregular, hence the sensitivity of direct observation of larvae decreases considerably. Therefore, in these situations, more sensitive and specific diagnostic tests are needed. The

new serological tests developed in recent years are only available in reference laboratories.⁵

The aim of this study is to evaluate the usefulness of *S. stercoralis* serology for the diagnosis of probable strongyloidiasis in patients presenting with eosinophilia and its role in the follow-up after treatment. This study includes both immunocompetent and human immunodeficiency virus (HIV)-infected patients.

PATIENTS AND METHODS

Study population, data collection, and objectives. Prospective observational study performed at the Infectious Diseases Department of the Vall d'Hebron Teaching Hospital (HUVH), a tertiary hospital included in the International Health Program of the Catalan Health Institute (PROSICS Barcelona, Spain). All patients with eosinophilia attended at the Infectious Diseases Department from January 2010 to December 2012 were included. Eosinophilia was defined as eosinophil cell count ≥ 500 cells/mm³ and/or a percentage $\geq 7\%$. Clinical and epidemiological data were collected: age, gender, country of origin, time since arrival to our country, epidemiological risk factor (immigrant, traveler), HIV infection, CD4+ cell count, and absolute and relative eosinophil cell count. The study protocol was approved by the institutional review board of the hospital and informed consent was obtained from all patients.

The endpoints of the study were to determine the percentage of patients with eosinophilia with positive *S. stercoralis* serology and without other alternative causes of eosinophilia, and to evaluate the usefulness of the serology in the follow-up of these patients after 6 months of specific treatment. Cure was defined as no detection of larvae and a negative serology 6 months after treatment. On the basis of previous studies, response to treatment was defined as negative serology 6 months after treatment or when by enzyme-linked immunosorbent assay (ELISA) the optical density (OD) ratio of post-treatment to pretreatment decreased to 0.6.^{6,7}

Diagnostic protocol. Stool samples from three different days were collected in recipients containing 10% formol saline from all patients. Microscopic examination was performed using direct techniques (saline and iodine wet mounts) and after concentration techniques using Ritchie's formalin-ether technique.

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Auramine stain for *Cryptosporidium* and *Isospora* detection was also performed in patients with HIV infection. Specific fecal culture for *S. stercoralis* larvae (agar plate culture with fresh stools) was performed when possible.

Schistosoma mansoni serology (ELISA, Novagnot *Schistosoma mansoni* IgG, Siemens Diagnostics, Marburg, Germany) and investigation in a urine sample for ova detection were performed in all patients coming from sub-Saharan Africa. Other tests were performed depending on epidemiological data of patients and physician criteria (specific serological tests, filarial investigation).

Strongyloides stercoralis serology was performed in all patients at the Department of Parasitology of the National Center of Microbiology (Instituto de Salud Carlos III, Madrid, Spain). Detection of *Strongyloides* immunoglobulin G (IgG) antibody was performed using the kit *Strongyloides* IgG IVD-ELISA (DRG Instruments GmbH, Marburg, Germany), approved by the European Community. It includes microtiter wells coated with the soluble fraction of *S. stercoralis* L3 filariform larval antigen. In this study, we used a cut-off value of 0.200; the test was considered positive if the index (ratio of OD measure of the sample and 0.200) was > 1.1 . This methodology has been previously used by Bon and others⁸ with a sensitivity of 91.2% and a specificity of 93.3%.

Treatment and follow-up protocol. In patients with a helminth infection diagnosis different from strongyloidiasis, specific treatment was offered. Treatment of *S. stercoralis* infection was offered to all patients with confirmed diagnosis or suspected diagnosis (combination of eosinophilia, positive *S. stercoralis* serology, and the absence of other causes of eosinophilia). Based on current recommendations, ivermectin 200 mcg/Kg/day for 2 days was the treatment of choice.⁹ Follow-up of treated patients were performed through detection of anti-*Strongyloides stercoralis* IgG, eosinophil cell count, and microscopic examination of stool samples from three different days, after 6 months of completion of treatment.

Statistical analysis. Categorical data are presented as absolute numbers and proportions, and continuous variables are expressed as medians and ranges. The χ^2 test or Fisher exact test, when appropriate, was used to compare the distribution of categorical variables, and the Mann-Whitney *U* test for continuous variables. Mean changes in eosinophilia and OD index before and after treatment were assessed by the Wilcoxon signed-rank test. Results were considered statistically significant if the 2-tailed *P* value was < 0.05 . The SPSS software for Windows (version 19.0; SPSS Inc., Chicago, IL) was used for statistical analyses.

RESULTS

One hundred and forty-seven patients were included during the study period. Figure 1 shows the flow diagram of patients. The median age was 35 (18–76) years and 75 (51%) patients were male. Twenty-eight (18%) patients had HIV infection, with a median CD4⁺ cell count of 471.5 (8–957) cells/mm³. Two (1.4%) patients were Spanish travelers coming from India and Brazil, and seven (4.8%) patients were Spanish without previous travel to endemic area for strongyloidiasis. One hundred and thirty-eight (93.9%) patients were immigrants coming from different geographical areas: 99 (71.7%) patients from Latin America, 29 (21%) patients from sub-Saharan Africa, 8 (5.8%) patients from Asia, and 2 (1.4%)

FIGURE 1. Flow diagram of patients.

patients from North Africa. The most represented country was Bolivia (60 patients).

Overall, 89 (60.5%) patients had a positive *S. stercoralis* serology. Among them, *S. stercoralis* larvae were detected only in 15 (16.8%, or 10.2% of the whole study population) patients, and 9 (6.1%) patients had other cause of eosinophilia: 4 patients with allergic diseases, 2 patients with hookworm infection, 2 patients with schistosomiasis, and 1 patient with *Trichuris trichiura* and *Ascaris lumbricoides* infection.

Agar plate culture was performed in 84 patients, with a 5 (5.9%) positive result, all of them with positive direct microscopic examination.

From the 58 (39.5%) patients with negative *S. stercoralis* serology, 21 patients had an etiological diagnosis: 5 patients with toxocaríasis, 4 patients with *Dientamoeba fragilis* infection, 2 patients with hookworm infection, 2 patients with schistosomiasis, 2 patients with anisakiasis, 2 patients with *Loa loa* infection, 2 patients with hypereosinophilic syndrome, and 2 patients with allergic diseases. All Spanish patients without previous travel to endemic area for strongyloidiasis had negative serology.

Therefore, 45 patients had a confirmed diagnosis: 15 patients with *S. stercoralis* infection, and 30 patients with other cause of eosinophilia. There were 102 out of 147 (69.3%) patients that had no alternative diagnosis; among these patients, 65 (63.7%) patients had positive *S. stercoralis* serology.

When comparing main findings between HIV-infected and non-HIV-infected patients, HIV-infected patients had a lower median value of absolute eosinophil count than

TABLE 1
Baseline epidemiological, analytical, and microbiological data of patients included in the study*

	Overall (N = 147)	Non-HIV-infected patients (N = 119)	HIV-infected patients (N = 28)	P value
Gender, male	75 (51%)	58 (48.7%)	17 (60.7%)	0.254
Age, years	35 (18–76)	35 (18–76)	35.5 (23–63)	0.726
Time since arrival, years	6 (0.1–34)	6 (0.2–34)	5.5 (0.1–25)	0.614
Absolute eosinophil cell count, cells/mm ³	1000 (300–4000)	1000 (437–4000)	700 (300–3600)	0.003
Relative eosinophil cell count, %	14.8 (6.2–38.5)	15.3 (7.1–38.5)	13.3 (6.2–35.6)	0.111
<i>Strongyloides stercoralis</i> larvae detection	15 (10.2%)	12 (10.1%)	3 (10.7%)	0.921
Positive <i>Strongyloides stercoralis</i> serology	89 (60.5%)	76 (63.9%)	13 (46.4%)	0.089
Optical density index in patients with positive serology (89 patients: 76 non-HIV and 13 HIV)	6.39 (1.20–18.60)	6.70 (1.20–18.60)	5.06 (1.30–9.90)	0.047

*Note: Data are reported as number (%) of patients or median value (range).

non-HIV-infected patients (700 cells/mm³ versus 1000 cells/mm³, $P = 0.003$), whereas there was no difference in the median value of relative eosinophil count. Although there was no difference in the percentage of positive serology between both groups, median of OD index in patients with positive serology was lower in the HIV-infected patients group (5.06 versus 6.70, $P = 0.047$). Among HIV patients, there were no differences in the median of CD4+ cell count between patients with positive and negative *S. stercoralis* serology (512 cells/mm³ versus 453 cells/mm³, $P = 0.836$). Baseline characteristics of patients are summarized in Table 1.

Patients with other diagnosed infections received specific treatment. These patients presented a reduction in the eosinophil cell count to normal levels.

Eighty patients (15 patients with confirmed diagnosis and 65 with suspected diagnosis of strongyloidiasis) received ivermectin 200 mcg/Kg/day for 2 days, from whom 32 patients completed the study protocol (48 patients were lost during follow-up or did not reach 6 months of follow-up when the study finished). Six months after treatment no patient presented with *S. stercoralis* larvae in the stools, 26 (81.3%) patients reached the response to treatment criteria, from which 11 (34.4%) patients fulfilled the cure criteria. As Figure 2 shows, there is an important decrease both in the OD index of serology and eosinophil cell count 6 months after treatment. Median of absolute eosinophil cell count pre-treatment and 6 months after treatment were 1,050 (575–4,000) cells/mm³ and 200 (100–700) cells/mm³, respectively ($P < 0.001$); median of OD index of serology pre-treatment and 6 months after treatment were 7.01 (1.65–18.60) and 1.38 (0.16–7.78), respectively ($P < 0.001$).

DISCUSSION

Eosinophilia is a common diagnosis among immigrants and travelers; nevertheless, the etiologic investigation is challenging, and often the cause of eosinophilia remains unknown.¹⁰ *Strongyloides stercoralis* infection is one of the most frequent diagnoses in patients presenting with eosinophilia, and it has been increasingly diagnosed in non-endemic areas. However, the index of under-diagnosis is expected to be high, because most patients are asymptomatic.^{3,11}

Detection of *S. stercoralis* larvae is the gold standard test for the diagnosis of strongyloidiasis; however, it is not sensitive enough. In chronic infected patients there is high variability caused by the irregular and low output of the larvae in feces.^{12,13} This intermittent larvae excretion has implications

in the diagnosis of strongyloidiasis and follow-up after specific treatment. A single stool examination fails to detect larvae in up to 70% of cases. A repeated examination of stool samples increases the sensitivity of the test: Nielsen and others¹⁴ reported a sensitivity of 50% with three stool examinations and can approach 100% if seven stool samples are examined. In our study, only 15 out of 80 (18.7%) patients with eosinophilia, positive *S. stercoralis* serology, and no other cause for eosinophilia had positive larvae detection in feces. Although it has been reported that an increase in the sensitivity with the specific fecal culture, it did not show any benefit in our study compared with direct microscopic examination.¹³

Different serological techniques have been developed during the last two decades to improve the sensitivity in the strongyloidiasis diagnosis: indirect immunofluorescence microscopy, gelatin particle agglutination, immunoblot, and ELISAs. Among all the techniques, ELISAs are the most widely used, and seem to have the highest sensitivity and specificity. However, serology usually overestimates the burden of disease, because serology may remain positive after resolution of the infection and the presence of cross-reactivity with other helminth infections: filariasis, schistosomiasis, and hookworm or *A. lumbricoides* infection.¹⁵

To avoid this overestimation of strongyloidiasis diagnosis by serology, we did not consider positive results when other cause of eosinophilia was detected. Moreover, the average of time since arrival to our country of the immigrant patients was high enough (6 years) to rule out most of the other non-detected helminth infections (schistosomiasis may persist in patients after 10 years out of endemic areas, but hookworm or *A. lumbricoides* infection are usually eliminated after 3–4 years of leaving an endemic area).

Another important fact is that the seven Spanish patients with eosinophilia, but without having visited an endemic region for strongyloidiasis, had negative serology. Because of the lack of sensitivity of direct diagnostic techniques, there are a considerable number of patients with eosinophilia with no confirmed diagnosis, and a positive *S. stercoralis* serology allows offering a targeted empirical treatment with ivermectin. In our study, *S. stercoralis* serology has been useful, because it has helped us to treat our patients as a suspected strongyloidiasis (positive serology but no detection of larvae in feces) in 65 patients with eosinophilia and without other alternative diagnosis.

Another issue regarding serological diagnosis is the interpretation of results in immunocompromised patients, because sensitivity of serology may significantly decrease, as a result of reduced antibody production.¹⁶ Nevertheless, *S. stercoralis*

FIGURE 2. Optical density index and eosinophil cell count before and 6 months after ivermectin treatment.

serology has been proposed in different studies performed in non-endemic areas to be used as a screening test in immigrant HIV-infected patients.^{17,18} In our study population, there was no difference in the percentage of patients with eosinophilia who presented a positive serology between HIV-infected patients and non-HIV-infected patients.

Six months after treatment, 81.3% of patients reached the response to treatment criteria (negative serology or a ratio of post-treatment to pretreatment OD index < 0.6). Previous studies have evaluated the usefulness of *S. stercoralis* serology in the follow-up after treatment, using different drugs: albendazole, ivermectin, thiabendazole, and pyrimin pamoate. Serological cure ranged from 27% to 91.7% depending on cure

criteria, treatment protocol, and time of evaluation after treatment.^{8,9,19–23} Time of evaluation seems to be critical, and cure rate in our study could be higher if evaluation was at 12 months or over; Loufty and others²² reported a serological cure from 38.4% at 3 months to 91.7% at 18 months. Nevertheless, it is important to note that reduction in OD index does not necessarily indicate eradication of *S. stercoralis*, but a reduction in the total parasite burden. Even so, serology has shown to be useful in follow-up after treatment, because direct diagnosis is not sensitive enough.

New diagnostic tools have been developed in the last few years to solve the lack of specificity of serological techniques. Krolewiecki and others²⁴ showed that the use of a recombinant antigen applied to the luciferase immunoprecipitation system may reduce the cross-reaction with patients with filariasis, increasing the specificity of the test. An ELISA has been used to detect coproantigen of *S. stercoralis*, and different real-time PCRs have been designed, achieving a specificity of 100%.^{25–27} However, more studies are required to validate these tests.

Potential weaknesses of this study include the low number of patients who completed the study protocol and the fact that the diagnosis is not always based upon the presence of parasite in the stools. The high number of patients who were lost during follow-up could be explained because most immigrants come to Spain seeking opportunities and have great mobility; it stresses the importance of using a sensitive screening test to offer treatment to as many people at risk of strongyloidiasis as possible, even when the infection is only suspected.

In summary, *S. stercoralis* serology is a useful diagnostic tool both in the diagnosis of probable strongyloidiasis and follow-up after treatment, although specificity may decrease as a result of cross-reactivity. In our study, serology has been also useful in HIV-infected patients. New serological and molecular tools are being developed to solve the cross-reactivity issue, but larger and prospective studies are needed to validate them.

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