Real-Time Polymerase Chain Reaction Assay on Bronchoalveolar Lavage: An Alternative Method for Diagnosing Chronic Pulmonary Schistosomiasis?

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Abstract. Schistosomiasis causes mainly hepatic and genitourinary damage. Although lung nodules have been commonly described in acute phase, they are presumably underdiagnosed in chronic schistosomiasis. We previously reported a series of patients with chronic pulmonary schistosomiasis confirmed by the histological examination of the lung biopsies. In the present work, we retrospectively tested an in-house real-time polymerase chain reaction for *Schistosoma* (currently validated for diagnosis on stool and on urine) in the bronchoalveolar lavage (BAL) of a couple of those patients, and both resulted positive. The possibility of testing BAL with molecular methods targeting a wide spectrum of pathogens, including parasites, is appealing. Further studies are needed to validate this technique that might reduce unnecessary biopsies.

Schistosomiasis is a neglected tropical disease which can cause mainly hepatic and genitourinary damage, depending on the species.¹ Pulmonary nodules are considered common findings in the acute phase of the infection,^{2,3} whereas lung involvement in the chronic phase has been traditionally associated with pulmonary hypertension.^{4,5} We recently revised this scenario, in light of a series of patients with chronic schistosomiasis showing pulmonary nodules, representing a relevant proportion (about 5%) of all schistosomiasis cases diagnosed at our Center for Tropical Diseases (CTD).⁶ The main challenge in those cases was to establish a correct diagnosis, avoiding pulmonary biopsies. The characteristics of the six patients diagnosed with lung nodules due to chronic schistosomiasis have been described previously.⁶ The first four patients underwent both a pulmonary biopsy and bronchoscopy with bronchoalveolar lavage (BAL) to rule out other possible severe diseases such as tuberculosis (TB) or malignant nodules. The histological examination of the lung biopsies demonstrated the presence of schistosome eggs, thus permitting to establish the diagnosis. Therefore, for the following two patients we preferred a presumptive treatment with praziquantel, avoiding invasive procedures. However, the latter, empirical approach might not be feasible in some cases, for which an etiological diagnosis might be required (for instance, when a high index of suspicion for malignancies or TB is present). Bronchoscopy with BAL is less invasive than pulmonary biopsy and could be used as a first line-procedure, in particular for the diagnosis of TB. Also, a cytological evaluation of BAL for possible malignant cells can be performed. Testing BAL for possible parasitic causes of pulmonary nodules, namely schistosomes, would be useful for selected cases, such as the ones described previously.⁶

Aim of this study was a preliminary retrospective evaluation of the real-time polymerase chain reaction (PCR) method in use at the CTD for the diagnosis of schistosomiasis in the BAL of patients previously diagnosed with schistosome lung nodules. Unfortunately, BAL was available for only two of our four patients who were submitted to bronchoscopy. The patients' main characteristics are summarized in Table 1. The

patients were two newly arrived migrants from West Africa, both presenting hypereosinophilia, hyper IgE, positive serology for *Schistosoma* spp (*Schistosoma mansoni* enzyme-linked immunosorbent assay, Bordier Affinity Products SA), and presence of *S. mansoni* eggs in the stool. One patient had also *Schistosoma haematobium* eggs in the urine. Both patients presented many nodules in both lungs (the lung nodules on computed tomography-scan of patient 2 are reported in Figure 1). The histological examination of the lung biopsies revealed presence of *Schistosoma* eggs in both patients.

The BAL samples were centrifuged at 13,000 rpm for 10 minutes. Once the supernatant was discarded, 200 μ L of lysis buffer (Magna Pure LC, DNA Isolation Kit I, Roche Diagnostics, Mannheim, Germany) was added to the pellet within Phocine Herpes Virus 1 DNA (PhHV-1) as internal control. The samples were heated for 10 minutes at 95°C before being loaded into the automatic DNA preparation instrument (Magna Pure LC 2, Roche Diagnostics). The *Schistosoma* spp primers and probe used for real-time PCR were the same published by Obeng et al.⁷ which amplifies a 77-bp fragment of the internal-transcribed-spacer-2. The amplification of each DNA sample was performed in a 25- μ L reaction mixture containing PCR SsoAdvanced Universal Probes Supermix (Biorad

FIGURE 1. Lung nodules on computed tomography-scan of patient 2.

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TABLE 1
Main characteristics of the two patients with lungs nodules due to chronic schistosomiasis

Patient	Age	Country of origin	Comorbidities and/or symptoms	Eosinophils Absolute count (%)	IgE (UI/mL)	Urine exam	Stool exam	Lung biopsy	Number and max diameter (mm) of lung lesions at CT scan	Schistosoma-PCR on BAL
1	27	Mali	Pulmonary TB HBV hepatopathy Abdominal pain	750 (13.9)	4,950	–	<i>S. mans</i>	<i>S. mans</i> eggs	8;13	Positive
2	28	Ivory Coast	Abdominal pain Cough	790 (12.9)	2,930	<i>S. haem</i>	<i>S. mans</i>	<i>S</i> spp eggs	> 10;10	Positive

BAL = bronchoalveolar lavage; CT = computed tomography; HBV = hepatitis B virus; PCR = polymerase chain reaction; *S. mans* = *Schistosoma mansoni*; *S. haem* = *Schistosoma haematobium*; *S* spp = *Schistosoma* species; TB = tuberculosis.

Laboratories Inc., Hercules, CA), 2.5 µg of bovine serum albumin (Sigma-Aldrich, St. Louis, MO), 80 nM of each *Schistosoma* spp and PhHV-1 primer, 100 nM of the double-labeled specific probe for *Schistosoma* spp, 200 nM of the double-labeled specific probe for PhHV-1, and 5 µL of the DNA sample. The thermocycler used was set to give 3 minutes at 95°C, followed by 40 cycles, each of 15 sec at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C. Amplification and the related data analysis were performed with the CFX96 (Biorad). The real-time PCR output from this system consisted of a cycle-threshold (Ct) value (set at 200), representing the amplification cycle in which the level of fluorescent signal exceeded the background fluorescence, and indicating the parasite-specific DNA load in the BAL samples tested. Both samples resulted positive to the real-time PCR for schistosome, with Ct values of 32.7 and 27.5, indicating a consistently higher amount of *Schistosoma* DNA compared with the positive control (Figure 2). This finding further reduces the possibility of false positive results.

In light of the increasing migration flows to Europe, there is an increasing need for diagnostic tools that can help clinicians in the diagnosis of tropical/parasitic diseases.⁸ The possibility of testing BAL with molecular methods not only for TB, but also for other pathogens implicated in similar radiological presentations would be particularly useful, also in settings less specialized in tropical medicine. In fact, schistosomiasis is underdiagnosed, and schistosome pulmonary nodules might

be frequently misdiagnosed as TB lesions.⁹ To our knowledge, at the moment, there are no real-time PCR methods validated for the detection of schistosome infection on BAL.¹⁰ Although our study did not aim at the validation of this method, the results are encouraging, despite the small number of patients tested. It must be considered that both patients had many nodules, and it would be interesting to know whether real-time PCR would be positive also in presence of a single lesion caused by schistosome. Further studies are needed to validate this approach that might reduce misdiagnosis and unnecessary biopsies.

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FIGURE 2. Results of DNA amplification. RFU = relative fluorescence units. This figure appears in color at www.ajtmh.org.