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Diagnostic study on an immunochromatographic rapid test for schistosomiasis: comparison between use on serum and on blood spot from fingerprick

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ABSTRACT

Background An immunochromatographic rapid test (ICT; Schistosoma ICT IgG-IgM, LDBIO Diagnostics) demonstrated high sensitivity (96%) in the diagnosis of *Schistosoma mansoni* and *S. haematobium*. To date, the test has been validated for use on serum only, but in the absence of lab equipment, blood drop from fingerprick could be a useful option. This method is acquiring more interest because of the high flow of migrants rapidly moving across Italy and other European countries.

Objective The aim of this prospective study was to evaluate the use of ICT on whole blood obtained from fingerprick.

Setting Centre for Tropical Diseases (CTD), Sacro Cuore Don Calabria Hospital, Negrar, Verona, Italy.

Participants The inclusion criteria were African migrants aged ≥18 years with epidemiological risk of infection. The exclusion criteria were refusal to participate in the study and impossibility of execution of one of the two study methods, for any reason. Seventy of the 72 eligible patients completed the study, 79% of whom were male.

Interventions The ICT was performed twice for each included patient: one on blood drop (by the research nurses, in the ward) and one on serum (by staff of CTD lab). The primary outcome was the concordance between the two methods, assessed by Cohen's kappa.

Results Cohen's kappa was 0.45 (95% CI 27.0 to 63.6), indicating moderate agreement between the ICT on serum and the ICT on blood drop. Assuming the results on serum as reference standard for diagnosis, the sensitivity and specificity of ICT on blood drop were 55% (95% CI 40 to 69) and 93% (95% CI 79 to 98), respectively.

Conclusions The agreement between the two diagnostic methods is too low to support the alternative one. Implementation of the kit for using blood drop instead of the serum and/or further studies aimed to identify easy-to-use tests for schistosomiasis feasible outside referral centres for tropical diseases are needed.

Strengths and limitations of this study

- This is the first study to evaluate the use of an immunochromatographic rapid diagnostic test for schistosomiasis in African immigrants in Italy.
- The study highlights the need for reliable, rapid and easy-to-use diagnostic tests to improve access to diagnosis and treatment for schistosomiasis in immigrants coming from endemic to non-endemic countries.
- The population under study (immigrants observed at the hospital) might not be representative of the general population of African immigrants in Italy.

BACKGROUND

Schistosomiasis is a parasitic infection caused by fluke worms of the genus *Schistosoma*. The main species affecting humans are *Schistosoma mansoni*, *S. haematobium*, *S. japonicum* and *S. intercalatum*. According to estimates, at least 200 million people are infected worldwide, most of them in sub-Saharan Africa.[1 2] Risk estimates of infection based on geostatistical modelling showed the highest prevalence in Mozambique (52.8% (48.7–57.8)), followed by Benin (36% (26.9–43.9)), Sierra Leone (35% (28.9–42.1)) and Mali (34.2% (31.3–37.1)).[3]

Most of the reported infections are clinically mild or even asymptomatic for a long time. However, chronic infections can lead to severe complications, mainly involving the urinary tract (haematuria, hydronephrosis, squamous bladder cancer) and the gastrointestinal tract (mucosal granulomatous inflammation, pseudopolyposis, microulceration, bleeding, hepatosplenomegaly, portal hypertension), according to the *Schistosoma* species.[4]

Despite the clinical relevance of this infection, and the potentially increasing number of cases present in most European countries due to migration flows,[5] schistosomiasis has

been not included in national screening programmes targeting migrants arriving from endemic countries to Europe. However, some recommendations in this sense have been recently proposed.[6 7] Authorities and health workers who are not used to dealing with these specific conditions are often simply unaware of the burden of this and other neglected tropical diseases. Moreover, control programmes tend to target infections perceived as a potential threat to the hosting populations, as is the case of tuberculosis and other communicable diseases.[8] However, the outbreak of schistosomiasis in Corsica, France, due to the colonisation of freshwater snails diffused along the Cavu Riverbank, reminded us that local transmission of imported parasitic infections is improbable but not impossible.[9]

The estimation of the real burden of schistosomiasis has been hampered by the lack of an adequate gold standard for diagnosis.[1] In fact, the detection of parasites' eggs in stool and urine samples using microscopic examination has low sensitivity. In turn this could underestimate the prevalence of infection. Hence, several indirect immunological tests have been implemented. Recently, a diagnostic study compared various techniques for the immunological diagnosis of schistosomiasis, including western blotting, ELISA, circulating cathodic antigen dipstick test and an immunochromatographic (rapid) test (ICT).[10] The latter demonstrated the highest sensitivity (96%), with a negative predictive value of 98%, and it might be the ideal tool for screening, at least in non-endemic settings. Other immunological rapid tests for different parasitic infections (mostly for malaria[11]) have been implemented for use with a blood drop from fingerprick, so that they can be performed in the field. In consideration of the constantly increasing migration flows from the poorest countries to Europe, sensitive screening tests that could also be used in the absence of minimal lab equipment (such as a centrifuge), such as primary healthcare centres for migrants, are appealing. Moreover, fingerprick instead of venipuncture would minimise the biological risk for the staff performing the test, besides being more acceptable for the migrants.

Objective

The aim of this study was to compare the results of the Schistosoma ICT IgG-IgM (LDBIO Diagnostics) performed on blood drops obtained from fingerprick with the ones obtained from the same test performed following the standard procedures and using the serum from venipuncture as the substrate.

METHODS

We performed a prospective study. Eligible patients were included once they gave their (written) informed consent.

Setting and participants

Eligible participants were all male or female African immigrants admitted for any cause to the ward of the Centre for Tropical Diseases (CTD) in Negrar from 3 April 2017 to 2 August 2017. Other inclusion criteria were age ≥18 years and exposure to epidemiological risk of infection (living in endemic areas, bathing in freshwater). Exclusion criteria were refusal to participate in the study and impossibility of execution of one of the two study methods, for any reason.

Interventions

A research nurse collected the blood samples from each patient included in the study, through venipuncture as well as through fingerprick. The two procedures were performed on the same day.

Procedures for the test on serum

Instructions for use were given by the manufacturer.[12] The blood sample was collected in an EDTA tube, labelled with the patient's code and then sent to the CTD lab. Once there, the sample was centrifuged and 30 µL of the obtained serum was added with a pipette to the ICT cassette, followed by two drops of eluent (supplied with the kit). The result was read between 20 and 30 min after the execution. Tests without appearance of the control band were considered uncorrected and then repeated. A positive test was defined by the appearance of a coloured band in the test field. Each result was read independently by two lab technicians, who were not aware of the result of the test performed from fingerprick on the same sample. In case of discordant results, a third technician was involved in the test reading. Each technician independently reported the result on a paper labelled with the patient's code.

Procedures for the test on blood drop

The blood obtained via fingerprick was dropped on the device. The research nurse performing the test and another investigator independently read the result and reported it on the case report form (CRF). They were both blinded towards the results obtained by the lab staff (who ran the test on serum in parallel). In case of discordant results, a second investigator was involved in reading the test. The time frame for reading and interpretation of the results was analogous to those used for the test on serum. Inconclusive results (given by absent or uncompleted migration of blood along the strip) were registered as such.

The principal investigator collected all CRFs, matched the information with the results obtained by the lab staff and entered all required data (including country of origin, date of birth, sex, date of execution from the test), anonymously, in a database (Excel file).

Analysis

The concordance among the results of the two procedures was assessed using Cohen's kappa coefficient. The results of Cohen's kappa were interpreted as follows: poor agreement=less than 0.20; fair agreement=0.20–0.40; moderate agreement=0.40–0.60; good agreement=0.60–0.80; and very good agreement=0.80–1.00.

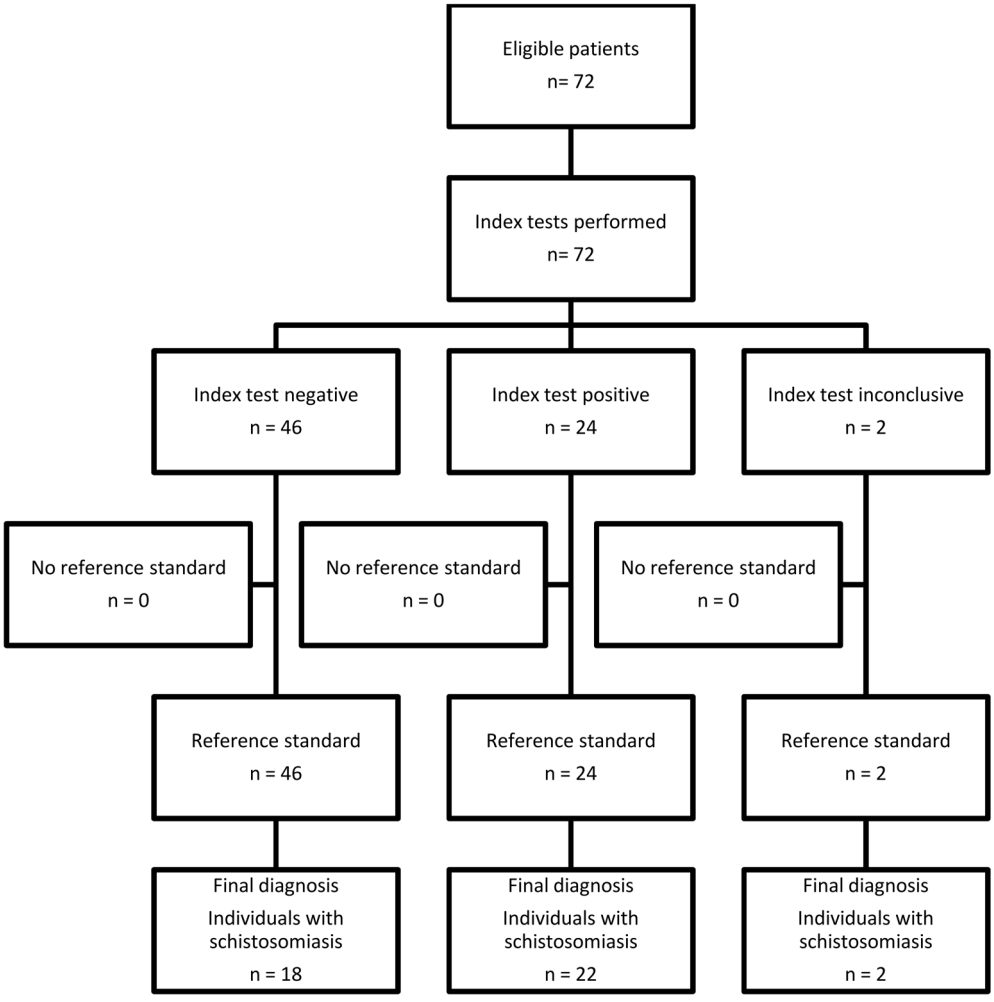

Figure 1 Study flow chart.

The expected prevalence of schistosomiasis in the study population was estimated at around 38%,[5] according to a recent study. The sample size was calculated using the 'R Studio' software (Open Source Ed), considering an alpha ≤0.05, study power ≥80% and a one-sided test. The number of patients to be included in the study was 69.

RESULTS

All eligible patients agreed to take part in the study. The study flow is presented in figure 1. The median age of participants was 24 years (IQR: 20.5–30.5), and 57/72 (79%) eligible patients were male. Countries of origin are reported in table 1.

In two cases (one patient from Mali and one from Côte d'Ivoire), the fingerprick test gave an inconclusive result (incomplete blood migration), while the corresponding tests on serum were positive. These two patients were eventually excluded from the analysis; hence, 70 patients were included.

A third reader was required twice for the ICT performed in the lab and three times for the test performed via blood spot. In all cases, the reason for involving a third reader was the presence of a very weak positive band; the final result was given as positive in all cases.

Table 2 shows the results of the ICT performed with the two methods.

Discordant results were 20 out of 70 (28.6%), mainly due to negative 'fingerprick' tests that were positive on serum (18 cases) instead. Cohen's kappa was 0.45 (95% CI 27.0 to 63.6), indicating moderate agreement. Assuming the results on serum as the reference standard, the sensitivity and specificity of ICT on blood drop were 55% (95% CI 40 to 69) and 93% (95% CI 79 to 98), respectively. Four patients were HIV-positive. Two patients had discordant results, with one of them resulting positive on the test on serum and negative on the fingerprick test, and vice versa in the second case.

DISCUSSION

The test performed on serum identified more positive patients than the same test performed on blood drop. As a result, the alternative method showed low sensitivity, when compared with the validated method. This might be

Table 1 Countries of origin of the eligible patients

Country	Patients included, n (%)
Western Africa	
Burkina Faso	1 (1.4)
Gambia	2 (2.8)
Ghana	9 (12.5)
Guinea	3 (4.2)
Guinea Bissau	5 (6.9)
Guinea	1 (1.4)
Côte d'Ivoire	14 (19.4)
Liberia	1 (1.4)
Mali	8 (11.1)
Nigeria	8 (11.1)
Senegal	7 (9.7)
Sierra Leone	1 (1.4)
Togo	1 (1.4)
Eastern Africa	
Eritrea	3 (4.2)
Sudan	1 (1.4)
Central Africa	
Angola	5 (6.9)
Cameroon	2 (2.8)
Total	72 (100)

due to the composition of the nitrocellulose strip, which may not allow a proper flow of the blood drop and/or the development of the antibodies/antigen bond might be altered in the presence of proteins present in whole blood. However, it must be considered that, according to our previous diagnostic study,[10] the specificity of ICT on serum is not as high as the sensitivity (around 80%). Hence, an additional explanation for the lower number of positive results obtained by fingerprick might be that this method produces less false-positive results. Moreover, the prevalence of schistosomiasis was similar to the expected one (38%) when evaluated with the test performed via fingerprick (34.3%), while it was extremely high (57.1%) when considering the test on serum. Although this might support the possible higher specificity of the test on blood drop, it might also be caused by a relatively high proportion of patients who were admitted to the CTD ward for symptoms/complications due to schistosomiasis (however, this information was not specifically collected in this study). In any case, based on the results of this study, the performance of the two methods differed too much to support the use of fingerprick test. Moreover, it must be considered that two patients had to be excluded for inconclusive results, and in three cases a third reader was involved, meaning that the test was not of easy interpretation. Further evaluation, including more diagnostic assays for schistosomiasis, is required to clarify our findings. In the meantime, the manufacturer communicated with us the intention to modify the nitrocellulose strip in order to improve its use with blood drops. In the last years, an increasing number of migrants from Africa has accessed our centre for screening and diagnosis of different parasitic infections. Many of them are only temporarily present in Verona province, and it is often difficult to plan follow-up visits. This scenario is common in many other places in Italy. Pending cost-effectiveness studies defining whether it might be worth, in our setting, to administer empirical antiparasitic treatments according to the area of origin of migrants, we need to identify diagnostic tools that can produce rapid, reliable results.

The treatment for schistosomiasis is short term and well tolerated[4]; hence, it might be worth treating individuals with positive results on a screening test, even in the absence of a second, more specific exam. This could represent a first step towards an improved access to the diagnosis and treatment of schistosomiasis in migrant populations.

Table 2 Results of the immunochromatographic rapid test performed with the two methods

	Test on serum		
Test on whole blood	Negative	Positive	Total
Negative	28	18	46
Positive	2	22	24
Total	30	40	70

Study limitations

ICT has not been extensively evaluated in the literature; hence, further diagnostic studies are needed to confirm our previous findings.[10] Additionally, we did not assess the influence of different volumes of whole blood on the accuracy of the test used via fingerprick, because our aim was to reproduce the field conditions. The small number of patients included in each geographical subset did not permit exploration of a possible association between discrepant results and the country of origin. Further, we could not analyse the possible relationship between the results of the tests and previous treatment with praziquantel, as the latter data were not available. Finally, another limitation is related to the study population. It was composed of patients admitted to the hospital; hence, they might not be representative of the general population of African immigrants present in Italy.

CONCLUSIONS

The use of ICT test on blood drop from fingerprick did not demonstrate an adequate concordance with the validated method (test on serum). Implementation of the same kit using blood drop as the substrate and/or further studies aimed to identify tests for schistosomiasis feasible to be performed outside referral centres for tropical diseases are needed.

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Disclaimer The manufacturer did not have any role in the design of the study, in the collection, analysis and interpretation of data, and in writing the manuscript.

Competing interests None declared.

Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Ethics approval The protocol (online supplementary file 1) received ethical clearance from the competent ethics committee (Comitato Etico per la Sperimentazione Clinica delle Province di Verona e Rovigo) on 14 March 2017 (study protocol number 13043).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The study data set is available on request to the corresponding author.

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