Detection of schistosome circulating antigens CCA and CAA: diagnostic test interpretation and application

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Appendix

SUMMARY

Schistosomiasis is a parasitic infection caused by flatworms (trematodes) of the genus *Schistosoma*. It is a neglected tropical disease, affecting mainly populations living in poverty without adequate sanitation. Treatment of all forms of schistosomiasis relies on one drug mainly, praziquantel (PZQ), due to its high efficacy and excellent safety profile. However, PZQ is ineffective against juvenile worms which may contribute to persistence of infection following treatment. Additionally, the estimated efficacy of PZQ is dependent on the diagnostic tool used.

The WHO 2030 roadmap for schistosomiasis calls for development of highly sensitive and specific field-deployable diagnostics to evaluate pre and post intervention. Diagnosis plays a key role in decision making on treatment of individual cases and to guide control programs such as the application of mass drug administration (MDA) in endemic settings. However, the lack of robust diagnostic tools impairs assessment and monitoring of the burden of schistosome infections which is crucial to identify infections and to interrupt transmission.

Due to the parasite's intravascular localisation, worms cannot be directly quantified in infected humans, therefore the diagnosis of *Schistosoma mansoni (S. mansoni)* infections are traditionally based on microscopy and determining the presence of schistosome eggs in the stool. However, egg microscopy lacks sensitivity, especially for detecting infections of low intensity such as in post-treatment settings. Thus, lack of egg detection does not necessarily indicate cure and it underestimates prevalence. Therefore, other methods of detection like worm-derived circulating cathodic antigen (CCA) in urine or circulating anodic antigen (CAA) in urine and serum, as well as serological tests have gained more attention.

While detection of the schistosome circulating antigens CCA and CAA has proven its diagnostic potential, there are still several questions to be solved. For example, it is unknown whether the reproductive maturity of the schistosomes affects the amount of antigen produced, or whether male and female worms have comparable regurgitation patterns and antigen production.

This thesis aims to explore and shed light on how to interpret schistosome CCA and CAA. In the beginning of this thesis, we demonstrated the importance of standardisation when reading the POC-CCA test scores (Chapter 2). In Chapter 3, we assessed the specificity of the POC-CCA when testing non-endemic urine samples. In Chapter 4 and 5, we explored schistosome circulating antigen-based methods in both endemic and traveller settings.

Lastly, in Chapter 6 we provided insights into the correlation between infection intensity based on a rodent model and the quantification of antigens both *in vivo* and from worm cultures. Deeper knowledge of CCA and CAA in different contexts could improve the application of current and future diagnostic methods.

Studies described in this thesis

In **Chapter 2**, we developed and evaluated an innovative approach for semi-quantitative interpretation of the POC-CCA cassettes, called G-scores with the aim to standardise and support POC-CCA visual scoring. The G-scores are a series of artificial cassettes containing inkjet-printed strips of different intensities in order to grade the POC-CCA test line on a scale of 1 to 10. We evaluated urines from a *S. mansoni* endemic area and the lines of the POC-CCA were visually compared against the G-scores. A significant positive correlation was observed between G-scores and eggs *per* gram of faeces. This proof-of-concept study demonstrates the usefulness of the G-scores for standardising the visual scoring of the POC-CCA urine strip assay. It also suggests the further assistance in dealing with issues like batch-to-batch differences and interpretation of traces.

Recent observations have raised concerns about possible reduced specificity of the POC-CCA, in particular in pregnant women (PW) and preschool aged-children (PSAC). However, there are limited data regarding the performance of the POC-CCA urine test in these groups because they have often been excluded in control programs based on MDA. In **Chapter 3,** we assessed the performance of the POC-CCA urine test in a non-endemic population, PW and children under the age of 4 that resided in the Netherlands, without any known exposure to a *Schistosoma* infection. The highest scores were found in the youngest babies, with an infant of 9 months being the oldest positive case. On measuring pH, it appeared that all POC-CCA strongly positive urines were acidic (pH range 5–5.5), whereas addition of pH-neutral buffer to a subsample reversed the false positivity. These findings suggest that the POC-CCA test has reduced specificity in PW and infants younger than 9 months, but that the false positivity might be eliminated by modifications in the buffers used in the test.

In **Chapter 4,** we determined the efficacy of a single versus four repeated treatments with PZQ on *S. mansoni* infection in school-aged children from Ivory Coast, using two different diagnostic tests (POC-CCA urine test and stool microscopy). Results showed a significant decrease in eggs (stool, microscopy) and CCA (urine, POC-CCA) after one treatment, but a quick resurgence if no additional treatment was given. Even after 4 repeated treatments, many participants were negative by microscopy but were still positive by POC-CCA. Results revealed discrepancies between POC-CCA and microscopy, reflecting the different life stages of the parasite. This also indicates that CCA-producing worms are still present

after multiple rounds of treatment and that PZQ may be considerably less effective than is concluded based on microscopy results. The data described in this chapter highlights the importance of further research on PZQ treatment efficacy in combination with accurate and sensitive antigen diagnostics.

In **Chapter 5,** we compared the added value of detecting urine and serum CAA as compared to serum antibody and PCR tests, in a prospective study based on Dutch travellers that have recently been to endemic areas. Our findings showed that only a small proportion of those exposed during travel established an active infection which could be diagnosed by the UCP-LF CAA assay. Following treatment with PZQ, serum CAA levels in these few individuals dropped to undetectable levels at 6 weeks and remained negative after 6 months. These findings confirmed that CAA detection shows an active *Schistosoma* infection and that PZQ did have an effect on light infections.

Whereas field studies in endemic areas provide insights from natural infections, animal models allow answering questions regarding CCA and CAA as diagnostic antigens which would remain unanswered in humans. **Chapter 6** described CAA and CCA excretion patterns by exploring a mouse model after exposure to 36 male-only, female-only and mixed (male/female) *S. mansoni* cercariae. Urine and serum samples from all mice were collected for antigen detection measurements at baseline, week 3, 6, 9 and 12 after infection. We found that male-only *Sm* worms regurgitate more CAA (*in vivo* and *ex vivo*) and more CCA (*ex vivo*) than female-only *Sm* worms. When measuring *ex vivo* worm cultures, we generally found more CAA than CCA produced *per worm* in both single-sex and mixed worms. Our study confirms that CAA levels reflect worm burden and allows detection of low-level single-sex infections. These findings suggest that knowledge of *Schistosom*a species excretion patterns of CCA or CAA is useful in order to appreciate test limitations in certain settings and facilitate decision making for public health interventions on the basis of antigen assay results.

Conclusion

Altogether, the studies in this thesis have addressed the interpretation of schistosome circulating antigen assays in endemic and non-endemic regions, supported by data obtained from an animal study. Different diagnostic value can be attributed to different assays within different contexts. We have shown that antigen excretion can differ between single-sex male or female schistosomes. Finally, this thesis highlights the importance of a better understanding of antigen excretion patterns by different species to support optimalisation of antigen-based diagnostics of schistosomiasis.