Cover Page

The handle http://hdl.handle.net/1887/39785 holds various files of this Leiden University dissertation.

Author: Downs, J.A.
Title: HIV and schistosomiasis : studies in Tanzania
Issue Date: 2016-05-31

CHAPTER 6

SCHISTOSOMIASIS AND IMPAIRED RESPONSE TO ANTIRETROVIRAL THERAPY AMONG HIV-INFECTED PATIENTS IN TANZANIA

Lucheri Efraim,[1] Robert N. Peck,[1,2,3] Samuel E. Kalluvya,[1,2]
Rodrick Kabangila,[1,2] Humphrey D. Mazigo,[1] Boniventura Mpondo,[1,2]
Heejung Bang,[5] James Todd,[6] Daniel W. Fitzgerald,[3] and Jennifer A. Downs[1,2,3]

[1]Department of Medicine, Catholic University College of Health and Allied Sciences, Mwanza, Tanzania; [2]Department of Medicine, Bugando Medical Centre, Mwanza, Tanzania; [3]Department of Medicine, Weill Cornell Medical College, New York, USA; [4]University of Dodoma, Dodoma, Tanzania; [5]Division of Biostatistics, Department of Public Health Sciences, University of California-Davis, California, USA; [6]Department of Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom

Schistosomiasis affects over 230 million people worldwide, 90% of whom live in sub-Saharan Africa (1). Data suggests helminthic infections like schistosomiasis may hasten HIV progression in co-infected patients (2,3). Helminths induce chronic immune activation, shifting from a T-helper cell type 1 (Th1) to type 2 (Th2) immune response. Th2- lymphocytes down-regulate cytotoxic effects of CD8+ T-lymphocytes, leading to an altered cytokine profile with increased viral replication (4–7). Studies have shown that treating ascariasis or filariasis improves CD4+ T-cell counts (CD4 counts) and viral loads in HIV-infected patients (8,9).

Little research has explored the specific interaction between schistosomiasis and HIV, but one study yielded concerning results. Antiretroviral therapy (ART)-naive HIV-infected patients with schistosome co-infection who were randomized to delayed anti-schistosome treatment with praziquantel after three months had larger increases in HIV RNA levels and greater declines in CD4 counts than patients treated immediately (10). Despite this possible interaction, schistosomiasis screening is not currently recommended for HIV-infected patients in many endemic countries, including Tanzania. Also, no study has yet assessed impact of schistosomiasis on ART response.

We hypothesized that schistosome infection may adversely affect HIV-infected patients' responses to ART. We conducted a retrospective cohort study to explore this issue at Bugando Medical Centre (BMC) near Lake Victoria in Tanzania, where schistosomiasis is hyper-endemic.

Methods

Study participants
This study was conducted from August-December 2011 at BMC's HIV clinic. HIV-infected adults who had taken ART for 6–15 months were enrolled serially. Patients who had received praziquantel since beginning ART, or who were currently receiving antituberculous therapy and therefore had another concomitant infection that could cause CD4 decrease (11), were excluded.

Data collection
Demographic information was collected by structured questionnaire. Baseline data (at ART initiation) was obtained from the patient database, including CD4 count, weight, and height.

At the time of enrollment and sample collection, we measured CD4 count by FACSCount system (BD Biosciences, San Jose, CA), height, and weight. We used

the World Health Organization (WHO) definition of immunological failure as either CD4 count falling below baseline or CD4 count persistently <100 cells/ mm^3 (11).

Patients provided single mid-day stool and urine samples. Kato-Katz stool smears were prepared using 41.7mg templates (Vestergaard Frandsen, Switzerland). Five slides were prepared from different sites of each stool sample, which has a reported sensitivity comparable to examination of specimens from different days (12). A trained parasitologist quantified *S. mansoni* eggs/gram.

Urine was examined microscopically and tested for circulating cathodic antigen (CCA) (Rapid Medical Diagnostics, South Africa). CCA, an antigen secreted into the bloodstream by adult schistosomes during active infection, is detectable in urine by rapid reagent test strip (13,14). Schistosome infection was defined as ova in stool or urine and/or a positive CCA test, a strategy shown to increase diagnostic sensitivity without compromising specificity for low-intensity *S. mansoni* infections typical of adult populations (15).

Data analysis
Logistic regression models (adjusting baseline CD4 count, which we call 'bivariate analysis,' and multivariate analysis adjusting for additional factors) were used to examine factors associated with immunological failure. In all models, we adjusted baseline CD4 count for more valid analyses, noting that the outcome, immunological failure, is defined as a function of baseline CD4 count. We used backward elimination, deleting variables with the largest p-value one by one, to reach a final parsimonious model including all factors with p<0.05.

We used analysis of covariance (ANCOVA) to compare CD4 count increases between groups with and without schistosomiasis while adjusting for baseline CD4 count (16). Two-sided 95% confidence intervals and p-values were used throughout. Data were analyzed using Stata IC/10.1 (College Station, Texas).

Ethics
Ethical approval was obtained from BMC and Weill Cornell. Patients diagnosed with schistosomiasis immediately received praziquantel (40mg/kg).

Results

Patient characteristics
Of 364 eligible HIV-infected outpatients coming to clinic during the study period, one had received praziquantel and 10 were being treated for tuberculosis.

Table 1. Baseline factors associated with immunological failure in HIV-infected adults after at least six months of antiretroviral therapy.

Factors	Immunological failure (n=25)	No Immunological failure (n=326)	Bivariable Analysis Odds Ratio	p-value	Multivariable Analysis Odds Ratio	p-value
Male gender	7 (28%)	96 (29%)	1.1 [0.4-2.7]	0.91		
Age in years	36 (32-49)	36 (31-43)	1.03 [0.99-1.08]	0.14		
Level of education*			0.4 [0.2-1.05]	0.062	0.3 [0.1-0.9]	0.036
No formal education	7 (28%)	24 (7%)				
Primary education	17 (68%)	270 (83%)				
Secondary education	0	18 (6%)				
College/higher education	1 (4%)	14 (4%)				
Occupation						
Business/professional	3 (12%)	34 (10%)	---	---		
Petty trading	13 (52%)	202 (62%)	0.7 [0.2-2.8]	0.52		
Unemployed	7 (28%)	68 (21%)	1.0 [0.2-4.2]	0.97		
Farming	1 (4%)	18 (5%)	0.5 [0.05-5.3]	0.45		
Fishing	1 (4%)	4 (1%)	2.3 [0.2-28.7]	0.36		
CD4 count (cells/μL) at time of ART initiation**	258 (189-298)	168 (73-239)	1.006 to 1.007	0.002 to 0.004	1.005 [1.001-1.009]	0.010
BMI (kg/m^2) at time of ART initiation	22.6 (20.8-24.4)	21.5 (19.5-23.8)	1.1 [0.98-1.2]	0.13	1.12 [1.01-1.24]	0.035
WHO clinical stage at time of ART initiation	3 (2-3)	2 (2-3)	0.9 [0.5-1.5]	0.72		
Days since ART initiation	336 (225-380)	333 (265-373)	1.0 [0.99-1.01]	0.91		
Schistosome infection	15 (60%)	82 (25%)	3.9 [1.6-9.1]	0.002	4.6 [1.9-11.2]	0.0009

Of the remaining 353 patients, 351 provided written informed consent, urine, and stool and were enrolled. Of these, 248 (71%) were females. The median age was 36 (interquartile range 31–43) years. Over 90% had primary school education or less, and >80% were unemployed or petty traders. Baseline CD4 count at ART initiation was 173 (76–249) cells/μL and baseline body mass index (BMI) was 21.6 (19.7–23.9) kg/m2. Baseline WHO clinical stages were: Stage 1--54 patients (15%), Stage 2—130 (37%), Stage 3—127 (36%), Stage 4—40 (11%). Patients had taken ART for a median of 338 (265–376) days.

Prevalence of schistosomiasis
Schistosomiasis was diagnosed in 97 patients (27.6%). All 97 were CCA-positive, and 46/97 had ova in urine or stool. All 46 of these had *Schistosoma mansoni*, and one had concurrent *S. haematobium*. All 46 patients with schistosome ova were CCA-positive, and 41/46 had low-intensity infections of <100 eggs/gram of stool.

Outcomes after ART
The median CD4 count change was +190 (104–303) cells/μL. Median BMI increase was +1.4 kg/m2. Twenty-five patients (7%) met ≥1 WHO criterion for immunological failure: 22 had CD4 counts below baseline and 7 had CD4 counts persistently <100 cells/μL.

Factors Associated with Immunological Failure
Table 1 shows univariate and multivariate analyses. In the multivariate model, education and baseline BMI were moderately associated with immunological failure (p=0.04), while schistosome infection was strongly associated (Odds ratio 4.6 [95% confidence interval: 1.9–11.2], p=0.0009).

Secondary analysis by ANCOVA, with change in CD4 count as a continuous outcome, showed that CD4 count increase on ART was significantly associated with schistosome infection, baseline CD4 count, and age in the multivariate,

---

Binary variables are reported with number and percent, and continuous variables are reported with median and interquartile range. For continuous variables/factors, odds ratios correspond to that for a one unit increase in variable (e.g., age 50 to 51).
*In regression models, education was modeled as a continuous variable.
**In all regression models, baseline CD4 was always adjusted as the outcome is defined as a function of baseline CD4. Thus, in bivariable analyses, results for baseline CD4 are summarized as a range rather than a single number.

parsimonious model. These effects were most strongly driven by schistosome infection status, with an estimated difference of 65.5 cells/μL in CD4 count change between those with and without schistosomiasis (p=0.0004). Unadjusted and adjusted mean CD4 count changes were +163 versus +226 cells/μL and +161 versus +227 cells/μL, respectively.

Discussion

Among these adult HIV-infected outpatients living in a schistosome-endemic area, nearly one-third had active schistosome infection. Odds of developing immunological failure were four times greater in patients with schistosome co-infection. To our knowledge this is the first study assessing the association between schistosomiasis and ART treatment failure. Our findings have major implications for ART management in millions of HIV-infected outpatients living in schistosome-endemic areas who are managed based on immunological and clinical criteria because viral load measurements are not routinely available.

Schistosome-infected patients also had significantly lower CD4 count increases on ART than schistosome-uninfected patients. More frequent immunological failure and smaller CD4 count gains in schistosome-infected patients could both be explained by chronic helminth-induced Th2-type immune activation, which may permit increased viral replication (4). Our work extends findings of a study in which Zimbabwean HIV- and schistosome-co-infected patients randomized to delayed praziquantel had larger HIV RNA increases and CD4 count declines than patients treated immediately (10). Other studies have suggested similar effects from other helminth infections, but not unanimously (8,9,17). Notably, other studies have not explored helminth infections' effects on patients receiving ART.

Our finding that schistosomiasis is both common and associated with immunological failure supports implementation of schistosomiasis screening at ART initiation. Unfortunately, stool and urine testing alone, particularly when done as the thin preparation of stool and unfiltered urine typical of many African clinical laboratories, has low sensitivity for detecting schistosome ova. Antigen tests including urine CCA used in this study may provide rapid, more sensitive screening for schistosomiasis, particularly since HIV-infected individuals may excrete fewer eggs (18,19). Further operational research is needed to determine costs, benefits, and optimal screening strategies.

In a previous study that compared baseline characteristics of schistosome-infected and uninfected HIV-positive patients, schistosome-infected patients had higher

baseline CD4 counts and CD4:CD8 ratios but comparable viral loads (6). Patients in that study were ARTnaive, as were ours during baseline investigations. Neither study observed other baseline differences between patients with and without schistosomiasis that might explain the higher CD4 counts. Schistosomiasis may cause distinct immunological alterations in peripheral blood CD4+ T-lymphocyte subsets, and these alterations may impair patients' responses to ART. Additional studies are needed to better-characterize peripheral blood CD4+ T-lymphocyte subsets in HIV and schistosomiasis co-infection.

The retrospective nature of our study is an ethically-necessary limitation since our hypothesis could not be studied prospectively. Based on the Zimbabwean study showing worse virological and immunological outcomes in HIV-infected patients with untreated schistosomiasis, it would not have been ethical to leave schistosomiasis untreated in patients initiating ART. Another limitation was our inability to test viral loads. Without virological data, we cannot determine whether schistosomiasis was associated with immunological failure alone or with concomitant virological failure. We plan further studies using viral load testing to explore this question.

In conclusion, nearly one-third of our Tanzanian HIV-infected outpatients had schistosome infection. Schistosome infection was significantly associated with immunological failure and poorer CD4 count gain following ART use. Untreated schistosomiasis may be a major cause of immunological failure among HIV-infected patients in schistosome-endemic areas. Further studies are needed to investigate whether this represents an immunological phenomenon or true virological failure, and whether screening and treatment for schistosomiasis among HIV-infected patients will improve response to ART. This is an urgent finding with major implications for ART management in resource-limited settings, where choices of antiretroviral medications are limited and success of patients on first-line ART must be maximized.

Acknowledgments

References

1. World Health Organization. Schistosomiasis Fact Sheet No 115 [Internet]. 2015 [cited 2016 Jan 8]. Available from: http://www.who.int/topics/schistosomiasis/en/

2. Bentwich Z, Maartens G, Torten D, Lal AA, Lal RB. Concurrent infections and HIV pathogenesis. AIDS. 2000 Sep 29;14(14):2071–81.

3. Joseph S, Jones FM, Laidlaw ME, Mohamed G, Mawa PA, Namujju PB, et al. Impairment of the Schistosoma mansoni-specific immune responses elicited by treatment with praziquantel in Ugandans with HIV-1 coinfection. J Infect Dis. 2004 Aug 1;190(3):613–8.

4. Bentwich Z, Kalinkovich A, Weisman Z. Immune activation is a dominant factor in the pathogenesis of African AIDS. Immunol Today. 1995 Apr;16(4):187–91.

5. Kullberg MC, Pearce EJ, Hieny SE, Sher A, Berzofsky JA. Infection with Schistosoma mansoni alters Th1/Th2 cytokine responses to a non-parasite antigen. J Immunol. 1992 May 15;148(10):3264–70.

6. Elliott AM, Mawa PA, Joseph S, Namujju PB, Kizza M, Nakiyingi JS, et al. Associations between helminth infection and CD4+ T cell count, viral load and cytokine responses in HIV-1-infected Ugandan adults. Trans R Soc Trop Med Hyg. 2003 Jan;97(1):103–8.

7. Clerici M, Shearer GM. A TH1-->TH2 switch is a critical step in the etiology of HIV infection. Immunol Today. 1993 Mar;14(3):107–11.

8. Walson JL, Otieno PA, Mbuchi M, Richardson BA, Lohman-Payne B, Macharia SW, et al. Albendazole treatment of HIV-1 and helminth co-infection: a randomized, double-blind, placebo-controlled trial. AIDS. 2008 Aug 20;22(13):1601–9.

9. Nielsen NO, Simonsen PE, Dalgaard P, Krarup H, Magnussen P, Magesa S, et al. Effect of diethylcarbamazine on HIV load, CD4%, and CD4/CD8 ratio in HIV-infected adult Tanzanians with or without lymphatic filariasis: randomized double-blind and placebo-controlled cross-over trial. Am J Trop Med Hyg. 2007 Sep;77(3):507–13.

10. Kallestrup P, Zinyama R, Gomo E, Butterworth AE, Mudenge B, van Dam GJ, et al. Schistosomiasis and HIV-1 infection in rural Zimbabwe: effect of treatment of schistosomiasis on CD4 cell count and plasma HIV-1 RNA load. J Infect Dis. 2005 Dec 1;192(11):1956–61.

11. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents 2010 revision. Geneva; 2010.

12. Berhe N, Medhin G, Erko B, Smith T, Gedamu S, Bereded D, et al. Variations in helminth faecal egg counts in Kato-Katz thick smears and their implications in assessing infection status with Schistosoma mansoni. Acta

Trop. 2004;92(3):205–12.

13. van Etten L, Folman CC, Eggelte TA, Kremsner PG, Deelder AM. Rapid diagnosis of schistosomiasis by antigen detection in urine with a reagent strip. J Clin Microbiol. 1994 Oct;32(10):2404–6.

14. Deelder AM, De Jonge N, Fillié YE, Kornelis D, Helaha D, Qian ZL, et al. Quantitative determination of circulating antigens in human schistosomiasis mansoni using an indirect hemagglutination assay. Am J Trop Med Hyg. 1989 Jan;40(1):50–4.

15. Van Lieshout L, Panday UG, De Jonge N, Krijger FW, Oostburg BF, Polderman AM, et al. Immunodiagnosis of schistosomiasis mansoni in a low endemic area in Surinam by determination of the circulating antigens CAA and CCA. Acta Trop. 1995 Mar;59(1):19–29.

16. Vickers AJ, Altman DG. Statistics notes: Analysing controlled trials with baseline and follow up measurements. BMJ. 2001 Nov 10;323(7321):1123–4.

17. Walson J, Singa B, Sangaré L, Naulikha J, Piper B, Richardson B, et al. Empiric deworming to delay HIV disease progression in adults with HIV who are ineligible for initiation of antiretroviral treatment (the HEAT study): a multi-site, randomised trial. Lancet Infect Dis. 2012 Dec;12(12):925–32.

18. Fontanet AL, Woldemichael T, Sahlu T, van Dam GJ, Messele T, Rinke de Wit T, et al. Epidemiology of HIV and Schistosoma mansoni infections among sugar-estate residents in Ethiopia. Ann Trop Med Parasitol. 2000 Mar;94(2):145–55.

19. Karanja DM, Colley DG, Nahlen BL, Ouma JH, Secor WE. Studies on schistosomiasis in western Kenya: I. Evidence for immune-facilitated excretion of schistosome eggs from patients with Schistosoma mansoni and human immunodeficiency virus coinfections. Am J Trop Med Hyg. 1997 May;56(5):515–21.