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Rationale and design of a screening study to detect schistosomiasis-associated pulmonary hypertension in Ethiopia and Zambia

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Abstract

Schistosomiasis is a major cause of pulmonary arterial hypertension (PAH) worldwide, but the prevalence and risk factors for schistosomiasis-associated PAH (SchPAH) development are not well understood. Schistosomiasis-associated hepatosplenic disease (SchHSD) is thought to be a major risk factor for PAH development. Herein, we describe our plans for prospectively screening SchHSD subjects for clinical evidence of PAH at two major academic medical centers and national referral hospitals in Addis Ababa, Ethiopia and Lusaka, Zambia. The screening study will primarily be conducted by echocardiography, in addition to clinical assessments. Plasma samples will be drawn and banked for subsequent analysis based on preclinical animal model rationale. If successful, this study will demonstrate feasibility of conducting prospective cohort studies of SchPAH screening in schistosomiasis-endemic regions of Africa, and provide initial data on clinic-based disease prevalence and potential mechanistic biomarkers underlying disease pathogenesis.

Edford Sinkala and Hanan Yusuf Ahmed are co-first authors.

Brian B. Graham and Joan F. Hilton are co-senior authors.

KEYWORDS
pulmonary hypertension, schistosomiasis, study design

INTRODUCTION

Schistosomiasis-associated pulmonary arterial hypertension (SchPAH), a neglected tropical disease, is a fatal complication of schistosomiasis—a disease that results from chronic and recurrent infection with the helminthic parasite *Schistosoma*. As defined by the World Health Organization (WHO), PAH represents Group 1 among five classifications of pulmonary hypertension (PH), with SchPAH being one of the major etiologies of PAH. One challenge in diagnosing SchPAH is distinguishing it from these related manifestations. By current criteria diagnosis of forms of PH requires invasive right heart catheterization (RHC),[1] which is only performed when there is a high suspicion of disease presence and therefore is not suitable for serial assessments, such as screening. A consequence of RHC-dependent diagnosis for patients is that PAH diagnoses occur after the disease is well established, thus precluding early interventions. A consequence for public health planning is the difficulty in accurately estimating SchPAH prevalence, especially in sub-Saharan Africa where *Schistosoma* species are endemic and the majority of cases occur. Despite beneficial use of preventative chemotherapy and mass drug administration, schistosomiasis is believed to affect about 200 million individuals worldwide[2–8] and to be underdiagnosed.[9,10] In turn, SchPAH is thought to affect 0.5% to 5% of those with a history of schistosomiasis.[11,12] Better understanding the epidemiologic distribution of SchPAH prevalence would support targeted screening efforts aimed at early detection and treatment of this disease. As a step toward population-based estimates of SchPAH prevalence, we seek to conduct a prospective cohort study to screen for evidence of existing or developing SchPAH at two clinical sites in sub-Saharan Africa where schistosomiasis is endemic.

Pathogenic mechanisms by which those with schistosomiasis can develop SchPAH are not well understood.[13] Infection with *Schistosoma mansoni*, the species most clearly associated with SchPAH, also causes severe pre-portal liver fibrosis in ~10%, termed schistosomiasis-associated hepato-splenic disease (SchHSD), which can be diagnosed and staged by abdominal ultrasound (US).[14] SchHSD causes portal hypertension but not cirrhosis and is thought to be a precursor to development of SchPAH in many individuals. The portal hypertension opens perihepatic shunts, with embolization of eggs in the systemic vena cava and in precapillary pulmonary vessels where they elicit a strong Type 2 inflammatory reaction and pathologic TGF-β signaling,[15] the latter likely representing a pathway to PAH that is shared across etiologies (Figure 1). This putative pathway supports the hypothesis that patients with SchHSD are at elevated risk for SchPAH, with expected prevalence of 8%–25%,[16] making them suitable targets for SchPAH screening in clinical settings. In addition, it suggests that selected biomarkers of inflammation can help to identify patients with pulmonary vascular pathology who are developing PAH.

Expertise and equipment to conduct RHC are sparsely available in the developing world. Instead, screening for PAH relies primarily on echocardiographic (echo) evidence,[17,18] which requires skillful conduct and interpretation but cannot reliably assess left heart filling pressures to distinguish PAH from other pulmonary hypertension (PH) diseases. We use the terms PH and PAH where appropriate to reflect the absence and presence, respectively, of formal measurement of pulmonary vascular resistance and left heart filling pressures. Because of the important need for noninvasive screening for PAH, many studies have evaluated the accuracy of echocardiography relative to RHC in diagnosing PAH. These include a systematic review of 32 studies of asymptomatic individuals at risk of PAH,[19] studies comparing the accuracy of specific echo quantities {TRV_{max}, IVC, PAP_{mean}, RVSP} with specific RHC quantities {PAP_{mean}, $PAP_{systolic}$} at varying thresholds,[20,21] and a review of 55 studies discussing improvements in accuracy through speckle tracking echocardiograms (STE).[22] We use the term RVSP as an echo-based estimate of RHC-measured $PAP_{systolic}$. Additionally, two small studies compared 2D-STE versus RHC findings in 34 advanced PAH patients[23] and in 36 individuals (27 with mild PH, 9 healthy).[24] Ikeda et al.[23] reported that peak systolic strain (PSS) of the right ventricular (RV) free wall can detect RV dysfunction in PH patients with mild to severe disease, and that RV PSS also is associated with prognosis.[25] Park et al.[24] showed that two STE parameters—global longitudinal strain of RV ($RVLS_{global}$) and global longitudinal strain of RV free wall ($RVLS_{FW}$)—strongly correlate with RHC parameters {PAP_{mean}, CI, PVR}, as well as with 6MWD and B-type natriuretic polypeptide (BNP) concentration. Furthermore, during follow-up $RVLS_{global}$ changes in parallel with changes in PAP_{mean} and PVR, and reflects changes in treatment.[26] These studies support the promise of

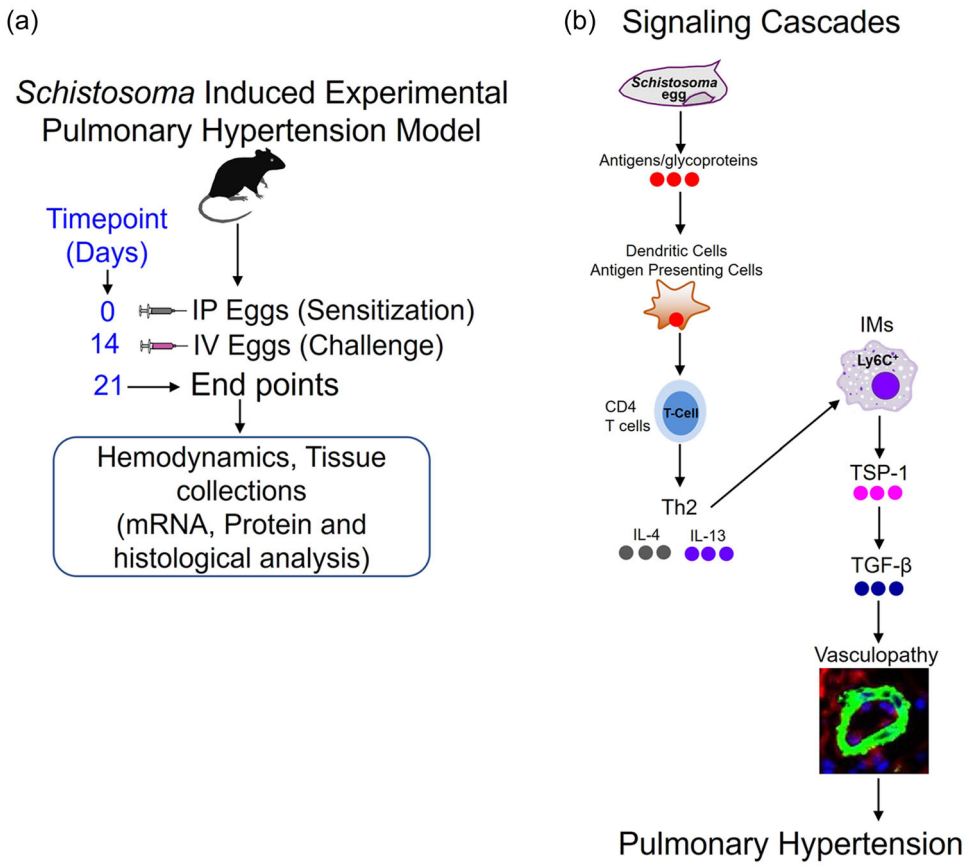

FIGURE 1 Preclinical model of schistosomiasis-PH, and mechanism of schistosomiasis-PH pathogenesis as indicated by preclinical studies. (a) The *Schistosoma*-PH mouse model uses intraperitoneal egg sensitization/intravenous egg challenge, followed by right heart catheterization and tissue analysis. (b) Live *Schistosoma* eggs in the lungs release glycoproteins such as omega-1, which is taken up by host dendritic and other antigen presenting cells (APCs). The APCs present the antigen to CD4 T cells, which become activated to a Th2 phenotype secreting proteins IL-4 and IL-13. This causes recruitment of Ly6c^{+} (classical) monocytes, which express the protein thrombospondin-1 (TSP-1) in a HIF2α-dependent manner. TSP-1 activates TGF-β (likely the isoform TGF-β1), resulting in pulmonary vascular disease

echocardiography to provide evidence of existing or developing PAH.

Considerable research has focused on predicting clinical worsening and mortality following RHC-based PAH diagnosis,[27–29] whereas few studies have been conducted to predict existing or developing PAH. REVEAL Lite 2 (R-Lite) is an example of a post-diagnostic prognostic score that was developed in RHC-diagnosed PAH cases to facilitate implementation in clinical settings. R-Lite is based on six noninvasive and modifiable variables from two domains[30]: blood levels {NT-proBNP, eGFR (calculated from creatinine, age, sex, race)} and functional metrics {6MWD, WHO functional class (FC), heart rate (HR), systolic blood pressure (SBP)}. We expect that the 14-point R-Lite score has a wide pre-diagnostic distributional range, which would support its use in risk-stratification for SchPAH development.

Ultimately, we seek to develop a clinically useful prediction model of SchPAH risk based on clinical data from multiple domains, including biomarkers of disease pathogenesis, echocardiographic features, and functional measures. We hypothesize that using a multidomain model to predict individuals' disease risk would be more accurate than using a single echo-based measure to define SchPAH disease presence (e.g., tricuspid regurgitation peak velocity (TRV) > 2.8 m/s,[31] or right ventricular systolic pressure (RVSP) = $4 \times \mathrm{TRV}^2 + \mathrm{RAP}$, with right atrial pressure (RAP) estimated from the inferior vena cava (IVC) diameter and the IVC collapsibility index[32]).

To obtain preliminary data in support of these goals, we propose to conduct a pilot prospective cohort study to screen for SchPAH development among patients with SchHSD at two academic medical centers that serve as national referral centers for schistosomal disease in Addis Ababa, Ethiopia and Lusaka, Zambia, in partnership with a Data Coordinating Center in San Francisco, USA.

STUDY SETTINGS AND CLINICAL/ RESEARCH EXPERTISE

Schistosomiasis is a tropical parasitic infection endemic to over 70 countries.[33] Of those, more than 80% are in sub-Saharan Africa,[34] with other regions of the world affected including Brazil, the Middle East, and Southeast Asia. *S. mansoni* and *S. haematobium* account for 90% of the worldwide disease burden, with *S. mansoni* causing hepatosplenic schistosomiasis and *S. haematobium* causing urofgenital schistosomiasis.[35]

Ethiopia and Zambia are among the sub-Saharan countries with high schistosomiasis prevalence.[36] Ethiopia is classified by the World Bank as a low-income country, and Zambia as a lower-middle income country. In Ethiopia, approximately 5 M have schistosomiasis (4.3% prevalence) out of a population of 115 M, with almost all cases due to *S. mansoni*, as *S. haematobium* is relatively rare in Ethiopia and only found in a few specific locations.[37,38] Zambia also has significant schistosomiasis, with a country-wide prevalence estimate of 36% (6.5 M) out of a population of 18 M.[39,40] However, both *S. haematobium* and *S. mansoni* are present in Zambia, and *S. haematobium* is more prevalent. Although not systematically studied, there is likely a substantial number of individuals in Zambia who are co-infected with these parasite species, depending on the specific geographic location studied. For example, a cross-sectional study of 111 school children 7–15 years old from the Chongwe and Siavonga Districts in central Zambia identified using urine PCR assays 77% of students infected with *S. mansoni*, 75% with *S. haematobium*, and 62% with both.[41]

One clinical site in the capital city of each country will participate in this study: Tikur Anbessa ("Black Lion") Specialized Hospital (abbreviated TASH) is operated by Addis Ababa University College of Health Sciences, and University Teaching Hospital-Adult & Emergency (abbreviated UTH-A&E) in Lusaka, Zambia. Both hospitals are the largest academic medical centers in their respective countries. Drs Ahmed and Sinkala, study co-PIs, will oversee protocol adherence and ensure data integrity at their respective sites. The study's Data Coordinating Center (DCC), staffed by biostatistical and pulmonary scientists, will be located at the University of California, San Francisco, USA.

Clinical site: Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia

TASH is the largest referral hospital in Ethiopia, with an 800-bed capacity and patients coming from throughout the country for care. TASH also is the largest teaching hospital for both clinical and preclinical training. It provides specialized clinical services not available in other public or private institutions in Ethiopia. The hospital is located in the capital city, Addis Ababa, which has five million inhabitants.

Dr. Ahmed, co-PI of this study, is the Chief of the Pulmonary Division at TASH, the only division providing pulmonary fellowship training in the country. The TASH pulmonary clinic is staffed by the pulmonary division three times per week, with three fellows, one attending and eight residents. As a growing service, the pulmonary function test clinic provides only spirometry tests.

The TASH Hepatology and Gastroenterology Clinic, part of the Division of Gastroenterology, holds clinic three times per week, staffed by dedicated fellows, internal medicine residents and attendings. Cases related to both gastroenterology and hepatology are referred for evaluation in these clinics. Schistosomiasis-hepatosplenic disease (SchHSD) is among the most common causes of portal hypertension presenting in these clinics, with an average of 200 annual cases before COVID-19.

The TASH echocardiography service is attached to the Cardiology clinic, and patients from both cardiac and other speciality clinics are referred for this service. The TASH catheterization laboratory is located on the same floor as the Cardiac Intensive Care Unit, with a well-equipped catheterization and recovery room.

Clinical site: University Teaching Hospital, Adult & Emergency (UTH-A&E), Lusaka, Zambia

UTH-A&E is a teaching and referral hospital under the auspices of the University of Zambia, School of Medicine (UNZA-SOM), the oldest university in Zambia. UTH-A&E is located in Lusaka, a city of 3.4 M inhabitants, and has a capacity of 800 beds. UTH-A&E and UNZA-SOM house the Tropical Gastroenterology and Nutritional Group (TROPGAN), founded in 1999, which is also constituted as a nongovernmental organization and not-for-profit company. Its primary focus is on translational science related to intestinal and liver disease and nutritional issues. TROPGAN has completed 20 research studies over the past 15 years.

Within TROPGAN is a Gastrointestinal/Hepatology procedure unit and a dedicated hepatology speciality clinic, overseen by Dr. Edford Sinkala (co-PI of this study) who is accompanied by three fellows. TROPGAN also has a dedicated laboratory, located on the same floor as the procedure area. The majority of cases seen in this clinic have schistosomiasis-related portal hypertension,

with about 5–10 each week pre-COVID (although more recently ~1 per week due to a reduction in outpatient care during the COVID pandemic). Routinely performed procedures at the clinic include diagnostic and therapeutic upper and lower endoscopy, liver biopsy, and aspiration.

The UTH-A&E Hemodynamic Unit, located in the Radiology Department, has one catheterization room and a 2-bed recovery room. There is one stationary echocardiography machine in the radiology department and two others in the Internal Medicine Department. The pulmonary disease service is equipped with pulmonary function tests and bronchoscopy facilities, and has a well-established clinic that runs 3 times per week.

Data coordinating center: University of California, San Francisco (UCSF), San Francisco, USA

A DCC located at UCSF will serve to coordinate and assist in the performance of the study. Dr. Brian Graham, co-PI on this study, has experience in the basic/translational pathophysiology of schistosomiasis-PH based on animal model studies (Figure 1), and will oversee the clinical and biomarker data collection and laboratory analyses. Prof. Joan Hilton, co-PI on this study, has expertise in biostatistics and study design, developed the study protocol, and will oversee study conduct and data analysis. The four co-principal investigators are collaborating on all phases of the study, from protocol specifications through data interpretation and reporting.

DESIGN OF PROSPECTIVE OBSERVATIONAL COHORT STUDY

To characterize the spectrum of pulmonary vascular disease in a longitudinal cohort of adult subjects at risk of SchPAH, this prospective observational study will accrue participants for 12 months and conduct follow-up visits targeting their 1-year anniversaries. Inclusion and exclusion criteria are designed to enrich the study sample for SchPAH by focusing on SchHSD patients in endemic areas who are free of other liver disease diagnoses and have no evidence of PAH etiologies other than SchPAH. Aside from blood sampling, the study will use noninvasive data collection methods, including echocardiography and clinical assessments. Data collected at study visits will be recorded by clinical personnel in a HIPAA-compliant REDcap database, accessible via mobile phones or desktop computers, and transmitted securely to the Data Coordinating Center at University of California, San Francisco.

Study protocol

We propose to identify, recruit, consent and study subjects with SchHSD who are being seen at the referral centers described above. Our study protocol defines the methods we will use to standardize data collection procedures across clinicians throughout the study, such as choice and calibration of equipment used to diagnose SchHSD and SchPAH, trainings, periodic analyses of data collected to examine inter-clinician variability and data completeness, specimen handling and lab QA/QC, and so forth, consent procedures, and the time required per patient to collect the data.

The study encounter, expected to last 2–3 h, will include the following steps. As needed, steps following consent could be scheduled at a later visit, as soon as possible.

(a) *Screening and consent*. Adult patients who have been diagnosed with SchHSD and are being seen in the referral centers described above will be approached for participation in this study by a member of the study team at the conclusion of a routine clinical visit. The inclusion and exclusion criteria (see below) will be reviewed to confirm eligibility, including prior abdominal ultrasound results to confirm the subject has evidence of SchHSD by standard criteria. If interested in participating, potential subjects will undergo consent by a member of the study team.

(b) *Documentation of history and data collection*. The study team developed a REDCap database which will be populated by study personnel at clinical sites and uploaded to servers at the Data Coordinating Center at UCSF for statistical review and analyses. A summary of the study data collected on each enrolled subject is in Table 1. Individuals who consent to participate will be interviewed for demographic, environmental, and medical history in the appropriate language by study personnel (Amheric in Ethiopia; Bemba or Nyanja in Zambia). The questions will identify schistosomiasis exposure and other potential causes of PH including family history of PH or personal history of anorexigen use, autoimmune disease, thromboembolic disease, congenital heart disease, COPD or other chronic lung diseases. We will also ask about personal history of hematuria, which is associated with infection by *S. haematobium*.

(c) *Medical records will be reviewed*. Recent laboratory data will be documented, including complete blood count and basic metabolic panel ordered at the preceding visit. Prior abdominal ultrasound results will be reviewed to confirm the subject has evidence of SchHSD by standard criteria. Current medication usage will be recorded, including any medications taken for PH.

TABLE 1 Planned recorded participant characteristics and study endpoints.

History
• HSD and PAH diagnosis dates; Clinic location; Clinician
• Home contacts, age, sex, primary language, education, occupation, home cooking fuel, tobacco/khat/alcohol use, source of drinking water, nutritional deficiency
• Comorbidities: sickle cell, malaria, anemia, viral hepatitis, CVD, COPD, diabetes, mitral valve regurgitation, esophageal/gastric varices, prior endoscopic studies
• *Schistosoma*: exposures by location and age {<5; >5 years}
• Prescribed therapies: PAH, schistosomiasis, other; drug name, adherence
Endpoint assessments
• Clinical: WHO FC, 6MWD, HR, BP, SpO2, Borg score, height, weight
• Echocardiography metrics: ∘ evidence of HSD: fibrosis ∘ evidence of PAH: PE, RAP (based on IVC), RA size, RV size, RVSP, RV strain, TAPSE, TRV
• Blood biomarkers: TSP-1, TGF-β, NT-proBNP, creatinine (to calculate eGFR)
• Risk score: REVEAL-Lite 2 {WHO FC, 6MWD, HR, SBP, NT-proBNP, eGFR}

Abbreviations: 6MWD, 6-min walking distance; BNP, B-type natriuretic peptide; BP, blood pressure; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; HR, heart rate; HSD, hepatosplenic disease; IVC, inferior vena cava; PE, pericardial effusion, RA, right atrium; RAP, right atrial pressure; RV, right ventricle; RVSP, right ventricular systolic pressure; TAPSE, tricuspid annular plane systolic excursion; TRV, tricuspid regurgitant velocity.

(d) *Echocardiography profile of study participants*. Individuals who consent to participate and are fully eligible will undergo an echo exam focused on the right side of the heart to diagnose PAH. Data that will be recorded include the tricuspid regurgitant jet velocity (TRV), IVC diameter and collapsibility index, RV size, RA size, LV size, LA size, estimated LV ejection fraction, and the presence and severity of stenosis or regurgitation of any of the valves. RVSP will be estimated. Patients with apparent congenital or structural heart disease or LV dysfunction on echo will be excluded from further study. Videos of key views with cardiac gating will be saved for later batch analysis to determine $RVLS_{global}$ and $RVLS_{FW}$.

(e) *Biospecimen collection*. Blood will be drawn from a peripheral vein into one 4 ml ETDA-containing tube (lavender top) and processed within 2 h. The sample will be centrifuged at 2000*g* for 10 min at room temperature, the plasma aliquoted into 500 μl volumes in separate tubes, and the tubes banked frozen at −80°C until analysis.

(f) *6-min walk test (6MWT)*. After the blood draw, subjects will undergo a 6MWT. We will perform before and after measurements of HR, SpO_2, BP, and Borg dyspnea score, in addition to recording distance walked (6MWD), per American Thoracic Society guidelines.[42]

Inclusion and exclusion criteria

We will define eligible participants with SchHSD by the following criteria (summarized in Table 2).

TABLE 2 Summary of inclusion and exclusion criteria.

1	Age at least 18 years old.
2	At least one of the following features consistent with schistosomiasis: **(a)** Prolonged exposure to an endemic region for schistosomiasis, and activities consistent with Schistosoma infection; **(b)** History of previous treatment for schistosomiasis; or **(c)** History or presence of *Schistosoma* eggs on stool examination (Kato-Katz testing) or on rectal biopsy.
3	Liver ultrasound with left lobe enlargement and/or periportal fibrosis.
4	No evidence of cirrhosis or other liver disease by history, or viral hepatitis.
5	Negative HIV test.
6	*After echo screening*,patients found to have significant structural heart disease such as congenital heart abnormalities or decreased left ventricle function will be excluded from further analysis.
7	Other exclusion criteria: pregnancy or prisoner.

1. *Age at least 18 years old*. The rationale is that: (1) SchPAH is thought to develop after longstanding schistosomiasis disease and is rarely diagnosed below the age of 18 years; (2) SchHSD is also rarely seen in patients younger than 18 years of age; and (3) other pediatric PAH etiologies are often different than adult PAH etiologies.

2. *History of schistosomiasis or reasonable exposure*. At least one of the following features consistent with schistosomiasis: (a) exposure to an endemic region for schistosomiasis, and activities consistent with *Schistosoma* infection; (b) history of previous treatment for schistosomiasis; or (c) history of or presence of *Schistosoma* eggs on stool examination (Kato-Katz testing) or on rectal biopsy. The rationale is that subjects should have a history of substantial and likely schistosomiasis exposure.

3. *Ultrasound evidence of SchHSD*. Liver ultrasound consistent with SchHSD, based on published guidelines on ultrasound screening criteria for schistosomal liver disease,[14] which is most commonly seen as either dense periportal fibrosis or enlargement of the left lobe of the liver. The rationale is that SchHSD is commonly diagnosed by ultrasound using standardized criteria, and while periportal fibrosis on liver biopsy is the gold standard for SchHSD diagnosis, this invasive test is impractical to conduct and relatively contraindicated in these individuals.

4. *No other known causes of liver disease*. No evidence of cirrhosis by ultrasound examination, or other liver disease by history such as viral hepatitis. The rationale is that we want to exclude other causes of portopulmonary hypertension which may potentially confound PAH pathogenesis.

5. *Negative HIV test*. All patients being seen at these clinics are screened for HIV, which is relatively uncommon in these populations. The rationale is that HIV can also contribute to PAH, and is excluded as a potentially confounding factor.

6. *No significant congenital or left heart disease*. After the subjects have consented and the echo study has been performed, patients found to have significant structural heart disease such as congenital heart abnormalities or decreased left ventricle function will be excluded from subsequent analysis. The rationale is there may be confounding of PH pathogenesis from congenital heart disease or left heart disease.

7. *No other standard reasons for exclusion*, such as inability to consent, current pregnancy, or a prisoner.

Research procedures added to routine clinical care

No medications or other interventions will be administered as part of this study. The patient and their physician will receive the results of the echocardiography and 6-minute walk tests. The physician may refer the patient to a pulmonologist as appropriate for further management if there is a concern for PAH.

Site biomarker processing and banking

The plasma will be processed at each site, with aliquoting and banking in −80°C freezers at each site until analysis. The freezers are located in the hospitals, and power outages are very rare. Due to restrictions of transport of specimens outside of each countries, the local teams will conduct assays using commercially available ELISA kits at each site for four proteins: creatinine (for eGFR, in R-Lite), NT-proBNP, TSP-1, and total TGF-β. When the clinical samples are assayed, we will use a standard curve and internal controls to maximize the accuracy of the results. Each site already has the equipment in place needed to perform these assays. The results from each assay will be uploaded to the same REDCap database, linked to each study subject.

Data coordinating center procedures

The DCC at UCSF has been hosting biweekly teleconferences as the study team has developed the protocol, survey instruments, and almost all of the regulatory paperwork. The DCC is currently assisting the clinical sites with regulatory paperwork requirements, and is maintaining the secure REDCap database which is shared by both sites. As the data collection begins, the DCC will review the data completeness and perform basic quality checks, and track screening and recruitment numbers by site. Before running the biospecimen assays, the DCC will review the laboratory assay protocols with the local teams by teleconference, and plan on conducting practice runs before processing any clinical samples. Analysis of the results will be performed using the statistical approaches outlined below.

Timeline

At the present time, we have completed the study protocol. We anticipate starting recruitment shortly, and will recruit subjects for 24 months duration. At 12 month anniversaries, we will schedule a follow-up visit on each participant, collecting plasma samples and echo and functional assessments. SchHSD subjects are typically seen in clinical follow up, although some patients who are stable may be discharged from care at these speciality clinics. If patients are diagnosed with PH, they may be

started on medication at the discretion of their clinical team, and treatment medications and clinical changes over time will be documented.

Statistical methods

Sample size

We project that over two-year accrual period beginning in late 2021, pools of 50 and 25 unique SchHSD patients per year will be available for recruitment at TASH and UTH-A&E, respectively, of whom 80% will consent to participate in this study. We propose to screen all SchHSD participants available at each site. Anticipating 60 SchHSD participants per year, we expect to identify at least 6 subjects with SchPH based on the echo-based screening measure, RVSP > 35 mmHg, discussed above. These data will be used for planning a future larger longitudinal cohort study. We will collect data over a 2-year study period from which we will estimate, for future studies, (i) the accrual rate, and how long it would take to enroll a range of target sample sizes, and (ii) the retention rate, and (iii) correlation among longitudinal outcomes, and corresponding precision of target sample sizes.

Preliminary summaries

All study data (Table 1) will be summarized using descriptive statistics suitable for continuous or categorical values. These summaries will estimate consent and 1-year retention rates, and will profile (i) subject candidates, and (ii) study enrollees. Among candidates we will characterize reasons for ineligibility: proportions of all who consent to participate who lack evidence of schistosomiasis exposure, or are excluded due to hepatic findings or alternative forms of PH. Among enrollees we will characterize baseline demographic characteristics, environmental risk factors, co-morbidities, and metrics from our three endpoint domains {echo, biomarkers, functional metrics}.

Primary endpoint: Prevalence of echo-diagnosed SchPAH (RVSP ≥ 35 mmHg)

Based on the literature reviewed above, we anticipate that the prevalence of SchPAH in a cohort of SchHSD subjects will be at least 10% overall. We also expect that it will vary among subgroups, such as by country (Ethiopia or Zambia), sex, and R-Lite scores. The prevalence estimate will depend on the at-risk SchHSD participants who meet eligibility criteria, and certainty about the estimate (e.g., 95% confidence interval [CI]) will depend on how many people we study, as well as variation in the estimate by subgroup characteristics. Using generalized estimating equations (GEE) with robust standard errors, we will estimate the proportion (95% CI) of screened patients with echo-diagnosed SchPAH, adjusted for the key risk factors listed above.

Secondary endpoints: Alternative measures of developing SchPAH

As the focus of our study is on screening, we are as interested in evidence of developing or progressing SchPAH as in disease presence. Thus, we also will use GEE models to estimate risk of this disease on an ordinal or continuous scale of RVSP values, not just the dichotomized scale. Furthermore, we will evaluate R-Lite and alternative echo metrics discussed in the literature (Table 1) as measures of SchPAH preclinical disease. We will evaluate the associations between continuous endpoints (e.g., RVSP and R-Lite or concentrations of biomarkers), using spline to allow nonlinear associations, as functions of key covariates. Within covariate levels, mean (95% CI) summaries of each endpoint will be illustrated via forest plots.

ACKNOWLEDGED LIMITATIONS OF THIS STUDY

Use of echocardiography and not RHC

Insofar as RHC is the gold standard in PAH diagnosis, and required to exclude left heart disease by measuring left sided filling pressures, we acknowledge that post-capillary PH may be included among our cases. For example, in a series of patients with schistosomiasis hepatosplenic disease in Brazil, 7.7% had PH by the former $PAP_{systolic}$ threshold (≥25 mmHg), with 4.6% having pre-capillary PAH.[43]

RHC is not widely available in locations where schistosomiasis is prevalent. At our two clinical sites, catheterization facilities exist but presently are only rarely used for RHC; the current standard of care in these settings is diagnosis of PH by echocardiography. In the future we are interested in developing the RHC capabilities but, practically speaking, alternative non-invasive and less expensive approaches need to be developed to screen for SchPAH in schistosomiasis-endemic settings.

Inability to distinguish causal from incidental SchPAH

A limitation in this field is the difficulty of establishing causality of prior schistosomiasis disease and liver complications, and the subsequent development of PAH. For example, it is possible that a patient may have been infected with schistosomiasis, and developed SchHSD, but then also developed IPAH. We anticipate that we will detect a relatively high prevalence of PH in these at-risk populations, much higher than would be anticipated from other PAH etiologies. Biomarker studies as we are proposing herein may identify subgroups or help clarify the precise disease definition, including clarifying pathogenic mechanisms linking exposure with subsequent disease.

Confounding of liver and lung disease

We hypothesize that the pre-portal fibrosis in SchHSD is a critical pathogenic trigger for the resultant pulmonary vascular disease found in SchPAH, but the two can also exist independently. We are not rigorously screening for other co-existing liver and lung diseases. Furthermore, PAH progression can independently lead to liver dysfunction.[44]

*S. haematobium*and *S. mansoni* co-infection

Although unlikely in Ethiopia, it is possible in Zambia that subjects with SchHSD (due to *S. mansoni* infection) also may have been infected with *S. haematobium*, and although not as well described *S. haematobium* also can cause PAH. We do not plan to systematically screen for complications of *S. haematobium*, but if there is a clinical history of hematuria we will document it, screen for active *S. haematobium* infection by urine study, and recommend evaluation for pelvic vein disease by ultrasound. If we find the prevalence of SchPH to be higher in Zambia than in Ethiopia, or higher in those with a history of hematuria, we will modify our study to more systematically screen for *S. haematobium* involvement.

Active or recurrent *schistosoma* infection

Most of the patients who are referred to these speciality clinics have been previously treated for schistosomiasis, and it is rare to identify actively infected cases. It is possible that active or recurrent infection may increase the risk of SchPAH pathogenesis. The most common screening method is a stool study, called Kato-Katz, which has low sensitivity and specificity. Generally, SchHSD patients on initial presentation are empirically treated with the anthelmintic praziquantel, which is typically effective after 1 dose, and associated with a low risk of side effects. We do not plan to test for active infection. The plasma samples that we are banking can be used for future assays to screen for seroprevalence (as in Farrag [45]) or for circulating parasite proteins or mRNA to screen for active infection.

Confounding from other environmental stimuli that can contribute to PH

High altitude (>2500 m) can cause PH,[46] and Addis Ababa is located at 2350 m: we are planning to document location of residence and correlate with altitude. Some Ethiopians use khat, a mild stimulant,[47] which could plausibly contribute to PH risk as other stimulants are likely causative of PH[48]: we will assess and quantify khat usage.

Problems with subject recruitment due to COVID-19

As was noted above, outpatient volumes are down considerably compared to pre-pandemic levels, for example at ~20% in Zambia. We are optimistic outpatient volumes will increase as diseases other than COVID-19 continue unabated, but future increases in COVID-19 case numbers may further suppress SchHSD outpatient visits. If recruitment lags, we anticipate recruiting inpatients with SchHSD who are admitted with intestinal bleeding complications, suspecting that these patients may have a more severe form of disease which is more likely to be complicated by PAH. We also will evaluate adding on additional sites, and have identified candidate sites in each location.

CONCLUSIONS AND FUTURE DIRECTIONS

We are excited to conduct this study, which started enrolling in November 2021. We anticipate that this multisite, multicountry study will clarify the prevalence of SchPAH in at-risk patients in African clinics. We hope that, by focusing on SchHSD subjects to enrich the patient population that we are screening for SchPH, will identify many who have evidence of PH based on

echocardiography criteria. We will also perform biomarker analysis to confirm that TSP-1/TGF-β signaling underlies SchPAH pathogenesis, as has been suggested by our preclinical studies.

In the future, we are interested in expanding to additional sites, potentially including more remote clinics in endemic areas, and other countries such as additional countries in sub-Saharan Africa and Brazil.

There are several limitations of our approach, which are related to the limited resources available in these clinical settings. Initial studies such as this one will establish feasibility and could lead to improved standard of care. Moving forward, we are interested in following patients over time, to determine the prognosis and natural history of SchPAH. Lastly, vasodilator therapy has been suggested to benefit those with SchPAH based on retrospective studies and small prospective cohorts, and ideally should be evaluated more systematically.

AUTHOR CONTRIBUTIONS

Wrote first draft: Edford Sinkala, Hanan Yusuf Ahmed, Brian B. Graham, and Joan F. Hilton. *Performed critical revisions*: Jean Pierre Sibomana, Michael H. Lee, Biruk Kassa, Rahul Kumar, Sula Mazimba, Amsalu B. Binegdie, Sydney Mpisa, and Kawana Wamundila. *Approval final draft*: all authors. Brian B. Graham and Joan F. Hilton take full responsibility for the article.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICS STATEMENT

This study was approved by the Addis Ababa University College of Health Sciences Institutional Review Board (Protocol number 067/21/IM), the University of Zambia Biomedical Research Ethics Committee (Protocol number 1400-2020), and the University of California San Francisco Human Research Protection Program Institutional Review Board (Protocol number 20-31348).

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REFERENCES

1. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, Williams PG, Souza R. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J. 2019;53(1).
2. Chitsulo L, Loverde P, Engels D. Schistosomiasis. Nat Rev Microbiol. 2004;2(1):12–3.
3. Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. Acta Trop. 2000;77(1):41–51.
4. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet. 2006;368(9541):1106–18.
5. Wilson MS, Mentink-Kane MM, Pesce JT, Ramalingam TR, Thompson R, Wynn TA. Immunopathology of schistosomiasis. ImmunolCell Biol. 2007;85(0818-9641 (Print)):148–54.
6. King CH, Galvani AP. Underestimation of the global burden of schistosomiasis. Lancet. 2018;391(10118):307–8.
7. Deol AK, Fleming FM, Calvo-Urbano B, Walker M, Bucumi V, Gnandou I, Tukahebwa EM, Jemu S, Mwingira UJ, Alkohlani A, Traoré M, Ruberanziza E, Touré S, Basáñez MG, French MD, Webster JP. Schistosomiasis—assessing progress toward the 2020 and 2025 global goals. N Engl J Med. 2019;381:2519–28.
8. Amaral RS, Tauil PL, Lima DD, Engels D. An analysis of the impact of the Schistosomiasis Control Programme in Brazil. Mem Inst Oswaldo Cruz. 2006;101(Suppl 1):79–85.
9. Mawa PA, Kincaid-Smith J, Tukahebwa EM, Webster JP, Wilson S. Schistosomiasis morbidity hotspots: roles of the human host, the parasite and their interface in the development of severe morbidity. Front Immunol. 2021;12:635869.
10. Colley DG, Andros TS, Campbell CH. Schistosomiasis is more prevalent than previously thought: what does it mean for public health goals, policies, strategies, guidelines and intervention programs?Infect Dis Poverty. 2017;6(1):63.
11. Ward TJC, Fenwick A, Butrous G. The prevalence of pulmonary hypertension in schistosomiasis: a systematic review. PVRI Rev. 2011;3(1):12–21.
12. Alves JL Jr, Gavilanes F, Jardim C, Fernandes C, Morinaga L, Dias B, Hoette S, Humbert M, Souza R. Pulmonary arterial hypertension in the southern hemisphere: results from a registry of incident Brazilian cases. Chest. 2015;147(2):495–501.
13. Sibomana JP, Campeche A, Carvalho-Filho RJ, Correa RA, Duani H, Pacheco Guimaraes V, Hilton JF, Kassa B, Kumar R, Lee MH, Loureiro C, Mazimba S, Mickael C, Oliveira R, Ota-Arakaki JS, Rezende CF, Silva L, Sinkala E, Ahmed HY, Graham BB. Schistosomiasis pulmonary arterial hypertension. Front Immunol. 2020;11:608883.
14. Richter J, Hatz C, Campagne G, Bergquist NR, Jenkins JM, UNDP/WorldBank/WHO Special Programme for Research and Training in Tropical Diseases. Ultrasound in schistosomiasis : a practical guide to the standard use of ultrasonography

for assessment of schistosomiasis-related morbidity. Second International Workshop, October 22–26, 1996, Niamey, Niger/ editors: J. Richter [et al.]. Revised and updated. Geneva: World Health Organization; 2000 (TDR/STR/SCH/00.1). Available from: https://apps.who.int/iris/handle/10665/66535
15. Kaonga P, Kaimoyo E, Besa E, Zyambo K, Sinkala E, Kelly P. Direct biomarkers of microbial translocation correlate with immune activation in adult Zambians with environmental enteropathy and hepatosplenic schistosomiasis. Am J Trop Med Hyg. 2017;97(5):1603–10.
16. Papamatheakis DG, Mocumbi AOH, Kim NH, Mandel J. Schistosomiasis-associated pulmonary hypertension. Pulm Circ. 2014;4(4):596–611.
17. Janda S, Shahidi N, Gin K, Swiston J. Diagnostic accuracy of echocardiography for pulmonary hypertension: a systematic review and meta-analysis. Heart. 2011;97(8):612–22.
18. Butrous G. Pulmonary hypertension: From an orphan disease to a global epidemic. Glob Cardiol Sci Pract. 2020;2020(1): e202005.
19. Finkelhor RS, Lewis SA, Pillai D. Limitations and strengths of doppler/echo pulmonary artery systolic pressure-right heart catheterization correlations: a systematic literature review. Echocardiography. 2015;32(1):10–8.
20. Venkateshvaran A, Seidova N, Tureli HO, Kjellström B, Lund LH, Tossavainen E, Lindquist P. Accuracy of echocardiographic estimates of pulmonary artery pressures in pulmonary hypertension: insights from the KARUM hemodynamic database. Int J Cardiovasc Imaging. 2021;37(9):2637–45.
21. D'alto M, Di Maio M, Romeo E, Argiento P, Blasi E, Di Vilio A, Rea G, D'Andrea A, Golino P, Naeije R. Echocardiographic probability of pulmonary hypertension: a validation study. Eur Respir J. 2022. 2102548
22. Kossaify A. Echocardiographic assessment of the right ventricle, from the conventional approach to speckle tracking and three-dimensional imaging, and insights into the "right way" to explore the forgotten chamber. Clin Med Insights Cardiol. 2015;9:65–75.
23. Ikeda S, Tsuneto A, Kojima S, Koga S, Nakata T, Yoshida T, Eto M, Minami T, Yanagihara K, Maemura K. Longitudinal strain of right ventricular free wall by 2-dimensional speckle-tracking echocardiography is useful for detecting pulmonary hypertension. Life Sci. 2014;111(1–2):12–7.
24. Park J-H, Kusunose K, Kwon DH, Park MM, Erzurum SC, Thomas JD, Grimm RA, Griffin BP, Marwick TH, Popović ZB. Relationship between right ventricular longitudinal strain, invasive hemodynamics, and functional assessment in pulmonary arterial hypertension. Korean Circ J. 2015;45(5):398–407.
25. Haeck ML, Scherptong RW, Marsan NA, Holman ER, Schalij MJ, Bax JJ, Vliegen HW, Delgado V. Prognostic value of right ventricular longitudinal peak systolic strain in patients with pulmonary hypertension. Circ Cardiovasc Imaging. 2012; 5(5):628–36.
26. Cho KI. Right ventricular longitudinal strain: a target indicator in the treatment of pulmonary arterial hypertension. Korean Circ J. 2015;45(5):362–3.
27. Humbert M, Farber HW, Ghofrani H-A, Benza RL, Busse D, Meier C, Hoeper MM. Risk assessment in pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. Eur Respir J. 2019;53(6):1802004.
28. Benza RL, Gomberg-Maitland M, Elliott CG, Farber HW, Foreman AJ, Frost AE, McGoon MD, Pasta DJ, Selej M, Burger CD, Frantz RP. Predicting survival in patients with pulmonary arterial hypertension: the REVEAL risk score calculator 2.0 and comparison with ESC/ERS-based risk assessment strategies. Chest. 2019;156(2):323–37.
29. Hoeper MM, Kramer T, Pan Z, Eichstaedt CA, Spiesshoefer J, Benjamin N, Olsson KM, Meyer K, Vizza CD, Vonk-Noordegraaf A, Distler O, Opitz C, Gibbs J, Delcroix M, Ghofrani HA, Huscher D, Pittrow D, Rosenkranz S, Grünig E. Mortality in pulmonary arterial hypertension: prediction by the 2015 European pulmonary hypertension guidelines risk stratification model. Eur Respir J. 2017;50(2).
30. Benza RL, Kanwar MK, Raina A, Scott JV, Zhao CL, Selej M, Elliott CG, Farber HW. Development and validation of an abridged version of the REVEAL 2.0 risk score calculator, REVEAL Lite 2, for use in patients with pulmonary arterial hypertension. *Chest*. 2021;159:337–46.
31. Galiè N, Humbert M, Vachiery J-L, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper MESC Scientific Document Group. ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur Heart J. 2016; 37(1):67–119.
32. McGoon M, Gutterman D, Steen V, Barst R, McCrory DC, Fortin TA, Loyd JE, American College of Chest P. Screening, early detection, and diagnosis of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. Chest. 2004;126(1 Suppl):14S–34S.
33. Coltart C, Whitty CJM. Schistosomiasis in non-endemic countries. Clin Med. 2015;15(1):67–9.
34. McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ, Zhou XN. Schistosomiasis. Nat Rev Dis Primers. 2018;4(1):13.
35. Lackey, EK, Horrall, S. Schistosomiasis. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing. 2021 [cited 2021 Oct 18]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK554434/
36. Adenowo AF, Oyinloye BE, Ogunyinka BI, Kappo AP. Impact of human schistosomiasis in sub-Saharan Africa. Braz J Infect Dis. 2015;19(2):196–205.
37. Negussu N, Mengistu B, Kebede B, Deribe K, Ejigu E, Tadesse G, Mekete K, Sileshi M. Ethiopia schistosomiasis and soil-transmitted helminthes control programme: progress and prospects. Ethiop Med J. 2017;55(Suppl 1):75–80.
38. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis. 2006;6(7):411–25.
39. Kalinda C, Chimbari MJ, Mukaratirwa S. Schistosomiasis in Zambia: a systematic review of past and present experiences. Infect Dis Poverty. 2018;7(1):41.

40. Kalinda C, Mutengo M, Chimbari M. A meta-analysis of changes in schistosomiasis prevalence in Zambia: implications on the 2020 elimination target. Parasitol Res. 2020;119(1): 1–10.
41. Hessler MJ, Cyrs A, Krenzke SC, Mahmoud ES, Sikasunge C, Mwansa J, Lodh N. Detection of duo-schistosome infection from filtered urine samples from school children in Zambia after MDA. PLoS One. 2017; 12(12):e0189400.
42. Holland AE, Spruit MA, Troosters T, Puhan MA, Pepin V, Saey D, McCormack MC, Carlin BW, Sciurba FC, Pitta F, Wanger J, MacIntyre N, Kaminsky DA, Culver BH, Revill SM, Hernandes NA, Andrianopoulos V, Camillo CA, Mitchell KE, Lee AL, Hill CJ, Singh SJ. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. Eur Respir J. 2014;44(6):1428–46.
43. Lapa M, Dias B, Jardim C, Fernandes CJ, Dourado PM, Figueiredo M, Farias A, Tsutsui J, Terra-Filho M, Humbert M, Souza R. Cardiopulmonary manifestations of hepatosplenic schistosomiasis. Circulation. 2009;119(11):1518–23.
44. Nickel NP, Galura GM, Zuckerman MJ, Hakim MN, Alkhateeb H, Mukherjee D, Austin ED, Heresi GA. Liver abnormalities in pulmonary arterial hypertension. Pulm Circ. 2021;11(4):20458940211054304.
45. Farrag A, El-Aroussy W, Zaghloul S, El-Guindy M, Yacoub M. Prevalence and severity of pulmonary hypertension in asymptomatic rural residents with schistosomal infection in the Nile Delta. Trop Med Int Health. 2012;17(1):112–8.
46. Xu X-Q, Jing Z-C. High-altitude pulmonary hypertension. Eur Respir Rev. 2009;18(111):13–7.
47. Alem A, Kebede D, Kullgren G. The prevalence and socio-demographic correlates of khat chewing in Butajira, Ethiopia. Acta Psychiatr Scand Suppl. 1999;397:84–91.
48. deJesus Perez V, Kudelko K, Snook S, Zamanian RT. Drugs and toxins-associated pulmonary arterial hypertension: lessons learned and challenges ahead. Int J Clin Pract Suppl. 2011;169:8–10.

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