BMJ Open

Evaluation of Artesunate-mefloquine as a Novel Alternative Treatment for Schistosomiasis in African Children (SchistoSAM): protocol of a proof-of-concept, open-label, two-arm, individually-randomised controlled trial

Clémentine Roucher,[1] Isabel Brosius ,[2] Moustapha Mbow,[3,4] Babacar Thiendella Faye,[5] Annelies De Hondt,[2] Bart Smekens,[2] Diana Arango,[2] Christophe Burm,[2] Achilleas Tsoumanis,[2] Linda Paredis,[1] Yven van Herrewege,[2] Idzi Potters,[2] Badara Cisse,[4] Souleymane Mboup,[4] Katja Polman,[1,6] Emmanuel Bottieau[2]

To cite: Roucher C, Brosius I, Mbow M, *et al*. Evaluation of Artesunate-mefloquine as a Novel Alternative Treatment for Schistosomiasis in African Children (SchistoSAM): protocol of a proof-of-concept, open-label, two-arm, individually-randomised controlled trial. *BMJ Open* 2021;**11**:e047147. doi:10.1136/bmjopen-2020-047147

► Prepublication history and additional supplemental materials for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2020-047147).

CR and IB contributed equally. KP and EB contributed equally.

Received 19 November 2020
Revised 04 March 2021
Accepted 19 March 2021

For numbered affiliations see end of article.

Correspondence to
Dr Isabel Brosius;
ibrosius@itg.be

ABSTRACT

Introduction Alternative drugs and diagnostics are needed for the treatment and control of schistosomiasis. The exclusive use of praziquantel (PZQ) in mass drug administration programmes may result in the emergence of drug resistance. PZQ has little activity against *Schistosoma* larvae, thus reinfection remains a problem in high-risk communities. Furthermore, the insufficient sensitivity of conventional microscopy hinders therapeutic response assessment. Evaluation of artesunate-mefloquine (AM) as a Novel Alternative Treatment for Schistosomiasis in African Children (SchistoSAM) aims to evaluate the safety and efficacy of the antimalarial combination artesunate-mefloquine, re-purposed for the treatment of schistosomiasis, and to assess the performance of highly sensitive novel antigen-based and DNA-based assays as tools for monitoring treatment response.

Methods and analysis The SchistoSAM study is an open-label, two-arm, individually randomised controlled non-inferiority trial, with a follow-up of 48 weeks. Primary school-aged children from the Richard Toll district in northern Senegal, an area endemic for *Schistosoma mansoni* and *Schistosoma haematobium*, are allocated to the AM intervention arm (3-day courses at 6-week intervals) or the PZQ control arm (single dose of 40 mg/kg). The trial's primary endpoints are the efficacy (cure rate (CR), assessed by microscopy) and safety (frequency and pattern of drug-related adverse events) of one AM course versus PZQ at 4 weeks after treatment. Secondary endpoints include (1) cumulative CR, egg reduction rate and safety after each additional course of AM, and at weeks 24 and 48, (2) prevalence and severity of schistosomiasis-related morbidity and (3) malaria prevalence, incidence and morbidity, both after 24 and 48 weeks. CRs and intensity reduction rates are also assessed by antigen-based and DNA-based diagnostic assays, for which performance for treatment monitoring is evaluated.

Ethics and dissemination Ethics approval was obtained both in Belgium and Senegal. Oral assent from the children and signed informed consent from their legal representatives was obtained, prior to enrolment. The results will be disseminated in peer-reviewed journals and at international conferences.

Trial registration number NCT03893097; pre-results.

Strengths and limitations of this study

- This study is the first large randomised controlled trial to evaluate artesunate-mefloquine (AM) as an alternative treatment for schistosomiasis.
- This study provides a comprehensive validation of a panel of top candidate diagnostic tools for monitoring antischistosomal treatment response.
- Promising field-applicable tools are evaluated as indirect markers of morbidity due to schistosomiasis before and after treatment.
- The particular inclusion and exclusion criteria of our study will not allow application of our results to all treatment indications.
- The potential impact of the use of AM in schistosomiasis control programmes on malaria cannot be determined from our study, given the very low malaria transmission in the study area.

INTRODUCTION

Treatment of schistosomiasis and the need for alternative drugs

Schistosomiasis is a parasitic helminth infection, disproportionally affecting children in poor rural areas.[1] It is a major public health problem, with an estimated global mortality of 24 072 in 2016, 21 151 of which (88%) was

in sub-Saharan Africa according to WHO.[2] The Global Burden of Disease Study 2016 found a prevalence of more than 180 million people worldwide accounting for almost 1.5 million years lived with disability (YLDs). Schistosomiasis is ranked in the top 10 YLDs in six sub-Saharan African countries.[3–5] The two major species *Schistosoma haematobium* and *Schistosoma mansoni* are responsible for organ-specific morbidity of the urogenital tract and hepatointestinal system, respectively, as well as anaemia, malnutrition and impaired physical and cognitive development.[6 7] Since the 1970s, treatment of schistosomiasis has relied almost exclusively on praziquantel (PZQ). The WHO-recommended 40 mg/kg single-dose regimen has demonstrated good tolerability and efficacy against adult worms, with cure rates (CRs) around 75% and egg reduction rates (ERR) above 85% for both species.[8 9] Schistosomiasis control is based on mass drug administration (MDA) of PZQ at regular intervals to at-risk populations, mainly primary school-aged children. This strategy—known as preventive chemotherapy—aims to reduce current infection and prevent the development of (severe) morbidity.

While PZQ has been successfully deployed for schistosomiasis control in many countries,[1 10] there are important limitations. It has a limited impact on reinfection, especially in high-transmission settings, due to its lack of therapeutic activity against larval stages,[11 12] and there is a risk of emerging drug resistance associated with its extensive use in monotherapy.[13–20]

Despite these limitations, new drugs with the same safety profile, pan-*Schistosoma* activity and affordability are not expected in the short term.[13] However, repurposing of existing drugs may provide a valid alternative;[21] two antimalarial drugs, artemisinin derivatives and mefloquine, have demonstrated in vitro and in vivo activity against *Schistosoma* spp.[22 23]

Artemisinin derivatives, such as artesunate and artemether, show excellent efficacy against schistosome larval stages and moderate activity against adult worms in animal models.[24] They also yield promising results as a chemoprophylactic agents[25 26] or as adjuvant therapy in combination with PZQ,[25] but CR obtained with artemisinin derivatives in monotherapy were inferior to that of PZQ (CR=33% on average).[25] Mefloquine has a strong activity against both schistosome larval and adult stages of *S. mansoni* in mice[27] and the combination of mefloquine and PZQ was found superior to either drug alone in mice models.[27 28] In contrast, the efficacy of mefloquine in monotherapy for human schistosomiasis was usually low (CR <50%) and evidence for its efficacy is still limited to a small number of clinical studies.[29 30]

Not only may the use of artesunate and mefloquine in combination be synergetic against schistosomiasis, the use of each of these drugs in monotherapy in malaria-endemic areas, poses a risk of inducing resistance of malaria parasites. The fixed drug combination artesunate-mefloquine (AM) is one of the WHO-recommended combination therapies for the treatment of uncomplicated malaria and has a well-established safety profile.[31] AM, therefore, appears a valuable candidate for repurposing against schistosomiasis.

Monitoring antischistosomal treatment efficacy and the need for alternative diagnostic tools

Historically, schistosomiasis diagnosis has relied on the microscopic detection of *Schistosoma* eggs in stool or urine by Kato-Katz (*S. mansoni*) or urine filtration (*S. haematobium*). Both methods are relatively straightforward, inexpensive and almost 100% specific. However, despite standard practice of repeated examinations to account for fluctuating egg counts, the insufficient sensitivity of microscopy results in an underestimation of the prevalence and an inaccurate assessment of treatment efficacy.[32–35]

With the current focus on MDA with PZQ for schistosomiasis control and elimination, the need for highly sensitive diagnostic tools is evident. A number of promising novel antigen-based or DNA-based detection assays need further evaluation as tools for monitoring antischistosomal treatment response:

DNA-based detection assay:

- Real-time PCR, to detect and quantify *Schistosoma*-specific DNA in stool and urine samples, is a powerful tool for the accurate diagnosis of schistosomiasis.[36] Specificity is virtually 100%, and sensitivities are equal to, or substantially higher than, conventional microscopy techniques.[32 37–39]

Antigen-based detection assays:

- The lateral flow immunochromatographic point-of-care (POC) test detecting *Schistosoma* circulating cathodic antigen (CCA) in urine (POC-CCA assay) is a rapid, user-friendly test designed for semi-quantitative field diagnosis of *S. mansoni* infection. Although some specificity issues are reported[40 41] and the interpretation of the trace results is disputed,[42 43] accumulated evidence suggests that a single POC-CCA test is more sensitive than conventional stool microscopy, especially for low infection intensities, such as after antischistosomal treatment.[32 44–46] The test has already shown its value for *S. mansoni* prevalence mapping in national control programmes.[46 47]
- The up-converting phosphor lateral flow assay detecting circulating anodic antigen (UCP-LF CAA) in urine is a user-friendly test for the quantitative diagnosis of *Schistosoma* spp at very low parasite loads.[48] Previous studies indicated that circulating antigen levels are a better indication and more stable measure of schistosome burden than egg counts.[49–51] Prevalence of *S. haematobium* and of *S. mansoni* infection based on UCP-LF CAA assay appeared threefold higher than when detected with a single urine filtration and significantly higher than when detected with a duplicate Kato-Katz, respectively.[52 53]

Monitoring morbidity after antischistosomal treatment

While ultrasonography is still the diagnostic tool of choice for detecting organ-specific lesions associated with chronic schistosomiasis,[54 55] field-applicable indirect tools

are increasingly used as sensitive surrogate markers of morbidity due to schistosomiasis.[56] Reagent strip tests that detect microhaematuria in urine, indicative of *S. haematobium* infection, have been suggested for monitoring urogenital schistosomiasis morbidity.[57 58] More recently, the point-of-care faecal occult blood (FOB) test was proposed to assess morbidity related to intestinal schistosomiasis.[59] Further research is needed to evaluate these tests as morbidity monitoring tools after antischistosomal treatment.

OBJECTIVES

The primary objective of the Evaluation of Artesunate-mefloquine as a Novel Alternative Treatment for Schistosomiasis in African Children (SchistoSAM) study is to evaluate the efficacy (using microscopy as a test of cure) and safety of a single course of AM. Our secondary objectives are (1) to evaluate the safety and cumulative efficacy of two additional courses of AM (at 6-week intervals each); (2) to determine the parasitological efficacy of single and repeated courses of AM, in terms of CR and ERR by *Schistosoma* spp and by infection intensity; (3) to assess the impact of repeated AM courses on schistosomiasis-related morbidity; (4) to determine the accuracy of novel schistosomiasis antigen-based and DNA-based diagnostic assays to monitor antischistosomal treatment response; (5) to determine the effect of repeated AM courses on prevalence and morbidity of *Plasmodium falciparum* infection and on incidence of clinical malaria; and (6) to monitor the potential emergence of mefloquine resistance and reduced artesunate susceptibility.

METHODS AND ANALYSIS

Participant and public involvement

Participants, being primary school-aged children, were not involved in the development of the research questions, study design our study conduct.

Study design

The SchistoSAM study is an open-label, two-arm, individually-randomised controlled non-inferiority trial. Participants in the control arm receive PZQ at baseline (single dose of 40 mg/kg, as recommended by WHO for the treatment of schistosomiasis), those in the intervention arm receive three courses of AM (4 mg/kg A+8 mg/kg M, once a day for three consecutive days, as used in the fixed-drug WHO-recommended combination treatment of uncomplicated malaria), at baseline and repeated two times at 6-week intervals. The 6-week interval between AM administrations was chosen because it corresponds to the time lapse for schistosome larval maturation. In addition, it should minimise the risk of cumulative mefloquine-associated toxicity. Follow-up, sample collection and drug safety assessment take place at regular predefined time intervals. At the end of the study, all children receive a standard dose of PZQ. This is done in coordination with the National Control Program. The study flowchart is represented in figure 1.

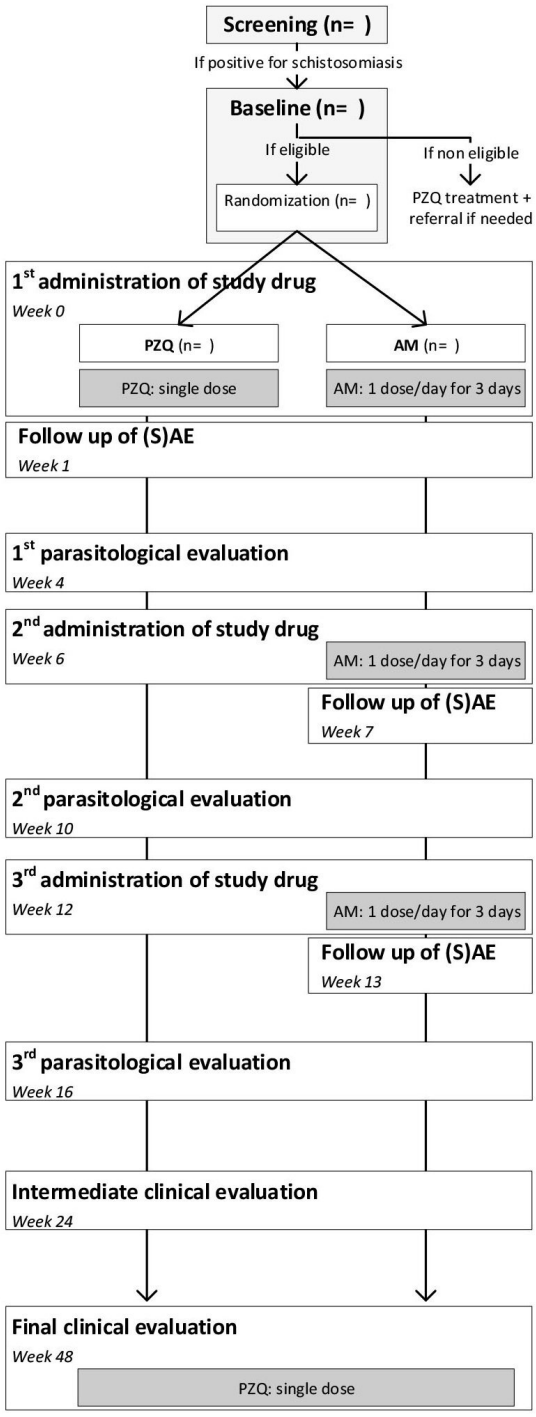

Figure 1 Study flowchart. AM, artesunate-mefloquine; PZQ, praziquantel; (S)AE, (serious) adverse event.

Study area and population

The study population consists of primary school-aged children (6–14 years old) from selected villages of the

Richard Toll district, located in the northern Saint-Louis region of Senegal. The area is coendemic for *S. mansoni* and *S. haematobium*, with a reported combined prevalence of >50% in the general population and up to 80% in school-aged children.[11 12] Malaria transmission is seasonal and very low (annual incidence <5/1000 inhabitants) and elimination is envisaged.[60] Hence, the area is well-suited to safely test AM drug efficacy against schistosomiasis.

Sample size

The sample size calculation was based on the hypothesis that the parasitological CR after the first course of AM (ie, primary endpoint) is non-inferior to PZQ with a power of 80%. Compared with the usual CR with PZQ (75%), a CR of 65% (maximum difference of 10%) is considered as clinically acceptable for non-inferiority of AM. For this purpose, the required sample size is 300 schoolchildren per arm, but to account for a 20% loss to follow-up, the total number of children to be randomised is 720 (360 per arm). Of note, the secondary endpoint, that is, superiority of repeated courses of AM to PZQ (CR of at least 85% after three courses) can be demonstrated with a similar power with this sample size.

Eligibility

Children are eligible for the trial if they comply with all of the following criteria:

- Age between 6 and 14 years.
- Enrolled in one of the selected primary schools in the district of Richard Toll.
- Confirmed infection with schistosomiasis at recruitment (ie, presence of *Schistosoma* spp eggs in urine and/or stool, as determined by microscopy).
- Written informed consent signed by parents/guardian(s) and an oral assent from the child.

Children who meet any of the following criteria are excluded from participation in the study:

- Past or present diagnosis of epilepsy or psychiatric illness.
- History of hypersensitivity to one of the study drugs (PZQ, mefloquine or artesunate/artemether).
- Chronic medication for any reason.
- Exposure to PZQ or artimisinin-based combination therapy (ACT) within 3 months prior to inclusion.
- Current febrile illness or clinical malaria at the time of inclusion.
- Any severe underlying illness, based on clinical judgement, including severe malnutrition, severe chronic. schistosomiasis or severe anaemia according to WHO criteria.
- Planned travel for more than 1 month within the first 4 months after enrolment.

Randomisation and blinding

A block randomisation schedule, stratified by the two *Schistosoma* sp, is prepared by the sponsor biostatistician, using SAS V.9.4 (SAS Institute). The individual randomisation number and treatment arm of each participant is stored in sealed envelopes, which are opened for each child by the study physician at enrolment. The laboratory technicians and the microscopists are blinded to treatment, but participants and clinical team members are not.

Study procedures

At inclusion, a written informed consent from the parents/guardians and verbal assent from the child is obtained. The parents/guardians are interviewed on the demographic characteristics and medical history of the child. The participant is then asked to give one stool and one urine sample to screen for schistosomiasis infection. *Schistosoma* spp positive children are subsequently invited for confirmation of eligibility. While ineligible children are offered standard treatment with PZQ, eligible children proceed to baseline clinical and laboratory evaluations. Symptoms are recorded through a pre-established questionnaire. The study physician performs a clinical examination, with specific attention to signs of anaemia, hepatosplenomegaly and stigmata of portal hypertension. Haemoglobin is measured and dried blood spot are collected for malaria detection. An experienced radiologist performs the ultrasound examination to assess schistosome-induced organ damage. In addition, children are asked to provide two urine and two stool samples, which are examined by a set of tests for the detection of *Schistosoma* infection and assessment of morbidity. Aliquots are preserved for further diagnostic workup.

Follow-up and sample collection take place in both arms 4 weeks after each round of AM treatment (week 4, 10, 16). At each time point, the children are asked to provide two stool and urine samples for analysis and aliquoting. A drug safety assessment is performed before each subsequent study drug administration, and on the first and fourth week after each treatment round, with 4 weeks marking the end of the adverse events (AEs) and serious adverse events (SAEs) collection period. The children are also asked about potential intercurrent malaria diagnosis. Whatman 3MM filter papers are made available at the local health centres, to collect blood spots for further analysis, from any study participant diagnosed with malaria between study visits. At weeks 24 and 48 after baseline treatment, intermediate and final clinical, sonographic and laboratory evaluations take place, equivalent to those at baseline.

A detailed listing of relevant study procedures at different time points is given in table 1.

Monitoring parasitic response after antischistosomal treatment

Microscopy

Parasitological diagnosis is determined by the microscopic detection of *Schistosoma* eggs in stool (*S. mansoni*) and urine (*S. haematobium*). For every stool sample, two microscope slides of 25 mg of faecal material each are prepared using the Kato-Katz method.

Table 1 Schedule of assessments

Time point	Screening	Baseline	Week 1	Week 4	Week 6	Week 7	Week 10	Week 12	Week 13	Week 16	Week 24	Week 48
Informed consent	X											
Eligibility check	X	X										
Demographics	X											
Medical history		X										
Clinical examination		X									X	X
Haemoglobin measurement		X									X	X
Microscopy (urine and stool)	X	X		X			X			X	X	X
POC-CCA		X		X			X			X	X	X
Urine reagent strip test		X									X	X
Faecal occult blood		X									X	X
Ultrasound		X									X	X
Randomisation		X										
Concomitant medication check		X	X	X	X	X	X	X	X	X	X	X
Symptom questionnaire		X	X	X	X	X	X	X	X	X	X	X
Malaria diagnosis		X*	X	X	X*	X	X	X*	X	X	X	X
Study drug administration												
AM arm		AM			AM			AM				PZQ
PZQ arm		PZQ										PZQ
(S)AE follow-up		X†	X	X	X†	X	X	X†	X	X		

*Malaria diagnosis is done on the first day of drug administration.
†(S)AE follow-up starts after dispensation of the study drug on the first day of drug administration.
AM, artesunate-mefloquine; POC-CCA, point-of-care-circulating cathodic antigens; PZQ, praziquantel; (S)AE, (serious) adverse event.

For every urine sample, a single slide is prepared after filtration of 10 mL urine through a filter of 12 µm pore size.[61 62] Results are expressed as the number of eggs per 25 mg of stool or 10 mL of urine. For quality control, 10% of the slides are re-read every day. In case of discordant results, those given by the most experienced microscopist are entered in the database.

Point-of-care-circulating cathodic antigens

For the POC-CCA assay (batch no.: 190411032, Rapid Medical Diagnostics, Pretoria, South Africa), 100 µL of urine is transferred into the cassette and allowed to migrate. Results are read after 20 min, using the semi-quantitative scoring method called 'G-scores'. An artificial score (1–10) is given according to the intensity of the test line compared with a set of 10 artificial cassettes with inkjet-printed bands of different intensity. As a semi-quantitative measure of intensity of infection, a score of 1 is considered as negative, scores of 2 and 3 as trace, scores of 4 and 5 as light infection (1+), scores of 6 and 7 as moderate infection (2+) and scores of 8–10 as heavy infection (3+).[63]

An aliquot of stool (3 g) and two aliquots of urine (1 mL each) are transferred in cryotubes and stored at −20°C. They are then shipped to the Institute of Tropical Medicine (ITM), Antwerp, Belgium, where additional diagnostic assays are performed:

Up-converting phosphor lateral flow assay for detection of circulating anodic antigen

For the UCP-LF CAA assay (UCA500), a maximum of 500 µL of filtrated urine is mixed with a buffer, concentrated and mixed again with CAA-specific UCP reporter conjugate solution. The LF strips are incubated therein and are afterwards scanned for UCP reporter signals with a fluorescent strip reader.[51 53 64 65] The results are reported quantitatively in pg/mL.

Multiplex real-time PCR

Real-time PCR analysis is performed on stool and urine aliquots to detect *Schistosoma*-specific DNA targeting the internal transcribed-spacer-2 sequence of *S. mansoni*, *S. haematobium* and *S. intercalatum* as described previously.[66 67] All PCR runs will include

a negative and positive control; furthermore, an internal control will be included and detected to control for PCR inhibition. For positive samples, the PCR output is a cycle threshold value, which is inversely proportional to the parasite-specific DNA load in the sample tested. For negative samples, there will not be an output value. Results from samples that show PCR inhibition will be marked as invalid.

Monitoring morbidity after antischistosomal treatment

Heme reagent dipstick test

A heme reagent dipstick test (Medi-Test Combi 5, Macherey-Nagel, Düren, Germany) is immersed into the urine, and the presence or absence of blood, glucose, leucocytes, nitrite and proteins is registered as displayed by the dipstick.

Faecal occult blood rapid test

A commercially available chromatographic test is used for the detection of FOB (Mission test, Acon Laboratories, San Diego, California, USA). A few milligrams of stool are mixed in a buffer from which three drops are transferred into a cassette and allowed to migrate. Results are read after 10 min and expressed as either trace, positive or negative.

Ultrasound examination

A qualified sonographer performs the ultrasound examination, using a portable machine (Samsung Medison SonoAce R3, Benetec, Belgium) set up in a private room in the village. Organ-specific lesions related to *S. mansoni* and *S. haematobium* are scored according to WHO guidelines.[68]

Haemoglobin measurement

A trained study nurse takes blood via a finger prick (about 200 µL) from each child to assess haemoglobin concentration/anaemia using a portable haemoglobinometer (HemoCue Hb 301, HemoCue, Ängelholm, Sweden). The given value is categorised according to WHO criteria.[69] Four separate drops of blood collected on Whatman 3MM filter paper in the field are stored at room temperature and shipped to ITM for the following analysis:

Monitoring for malaria infection and resistance markers

PCR for malaria detection and P. falciparum *genetic analysis*

A *P. falciparum* quantitative PCR (qPCR) targeting the var genes is performed on the dried blood spot samples.[70 71] On positive samples, pfmdr1 gene amplification, a marker of mefloquine/piperaquine resistance, is performed by qPCR.[72] Then, single nucleotide polymorphisms in pfmdr1 are detected through Taqman real-time PCR. Additionally, decreasing artesunate susceptibility is monitored by detecting mutations in the kelch propeller domain of the k13 gene of *P. falciparum,* using the Sanger sequencing approach.[73] Electrophoregrams are analysed on both strands with CEQ 2000 genetic analysis system software (Beckman Coulter, Villepinte, France), using PF3D7-1343700 as reference sequence. Isolates with mixed allele are considered as mutants.[74]

Treatment

Both the investigational fixed drug combination, AM and PZQ, are manufactured by the Indian company Cipla, and received WHO prequalification (WHO reference number: AM 25/50 MA078, AM 100/200 MA079, PZQ NT003). Each drug is administered according to study arm allocation in following posology: a single dose of 40 mg/kg PZQ at week 0 only, or 4 mg/kg A+8 mg/kg M in fixed drug combination daily over 3 (maximum 5) days (total dose of 12 mg/kg A+24 mg/kg M) at weeks 0, 6 and 12. A list of prohibited concomitant medications is distributed to the local health centres to prevent dispensation of such drugs to study participants. If non-study drugs were taken, the study physician evaluates whether AM administration can be pursued, considering possible interactions.

Adverse events

Intake of the study drug is observed directly, after which the child receives a light meal, and is monitored for 2 hours for any adverse reactions. If the child vomits within half an hour, the full dose is repeated, if it does so after 30 min, but within 2 hours, half of the dose is re-administered. If repeating the dose results in vomiting again, the treatment is discontinued.

During the follow-up visits, symptoms associated with adverse drug reactions and intake of concomitant medication are queried following a detailed questionnaire (ie, not spontaneously). The reported symptoms are graded as mild, moderate or severe, according to their intensity as perceived by the child and their impact on daily activities. Symptoms are reported as AEs, except when they are mild. In case of health problems between two successive field visits, participants are requested to contact the nurse of the local health centre or a community health worker, who in turn contacts the study physician, who reports an AE. A small stock of permitted emergency care medications is provided to the village healthcare centres.

AEs, SAEs and relatedness to the study drug are further defined and reported according to the Good Clinical Practice (GCP) guidelines in the 4-week period after each study drug administration.

Any SAE, whether or not deemed drug-related, is reported to the principal investigator, the coordinating investigator and the Data and Safety Monitoring Board (DSMB) within 24 hours. The sponsor will send a line listings of all reported SAEs to the ITM institutional review board (IRB) and the Antwerp University Hospital ethics committee (EC) on a yearly basis. In Senegal, the principal investigator will also report SAEs to the health authorities—through a level one public hospital—as required by local law.

Definitions and grading of symptoms and (S)AEs are detailed in a dedicated standard operating procedure, which is included in online supplemental additional files 1 and 2.

Endpoints

The primary study endpoints are:

- The CR as assessed by microscopy, after one AM course (week 4), compared with the standard PZQ regimen.
- The number of participants with drug-related (S)AEs in the first 4 weeks after one AM course administration, compared with the standard PZQ regimen.

The secondary endpoints are:

- The cumulative CR as assessed by microscopy, after the second and after the third AM administration (at weeks 10 and 16) and at longer term (at weeks 24 and 48), compared with the standard PZQ regimen. The total number of participants with drug-related (S)AEs in the 4 weeks after the second and third course of AM compared with the standard PZQ regimen and compared with a single course of AM.
- The ERR, as assessed by microscopy and the intensity reduction rate (IRR), as assessed by additional diagnostic assays, obtained after single and repeated course of AM (at weeks 4, 10, 16) and at longer term (at weeks 24 and 48), compared with the standard PZQ regimen.
- The CR, and the ERR and IRR, as assessed by microscopy and by novel diagnostic assays, respectively, by *Schistosoma* sp and by intensity of infection.
- The prevalence and severity of general and organ-specific schistosomiasis-related morbidity as assessed by clinical, laboratory and ultrasound examinations at baseline and at weeks 24 and 48.
- The sensitivity and specificity of the conventional and novel antigen-based and DNA-based diagnostic tests, to monitor antischistosomal treatment response.
- The prevalence of *P. falciparum*/malaria infection and the frequency and severity of anaemia at baseline and at weeks 24 and 48.
- The incidence of clinical malaria during the study until week 48.
- The frequency and pattern of *P. falciparum* resistance markers at baseline and at weeks 24 and 48.

Data management, monitoring and statistical analysis

Clinical, ultrasound and laboratory data are recorded and entered into REDCap, an ICH-GCP compliant data capture system. The data system includes password protection and internal validation checks to identify data that appear inconsistent, incomplete or inaccurate. Monitoring visits were planned; including site initiation and close-out, and two additional visits during the trial period, for source data verification.

All statistical analyses are detailed in a statistical analysis plan. For the efficacy analysis, both an intention-to-treat and a per-protocol approach are adopted, with the per-protocol analysis being the primary approach, as recommended for non-inferiority studies.[75] The primary hypothesis for non-inferiority is assessed by calculating the two-sided 95% CI for the difference in CR between arms. If it lies entirely above −10% then non-inferiority of the AM schedule is concluded.

The secondary analyses on the efficacy over time are assessed by calculating the two-sided 95% CI for the difference in proportions of cured subjects at different time points as well as by using mixed effects logistic regression models, one for each *Schistosoma* sp. Schistosomiasis infection at specific time points is used as the outcome variable, and treatment and visit as independent variables with a random intercept. The CR by *Schistosoma* infection intensity is analysed in a similar way, including that characteristic as covariate in the models. The analyses of ERR and IRR are performed using a linear mixed effects model with a random intercept separately for each *Schistosoma* sp.

Comparison of proportions of schistosomiasis-related morbidity is done using the χ^2 or Fisher's exact test, and comparison of continuous characteristics is done using the Wilcoxon rank-sum test.

Sensitivity, specificity, and positive and negative predictive value of both novel and conventional diagnostic tests are calculated together with 95% CI. In the absence of a true gold standard, these will be estimated by using a composite reference standard and by using latent class analysis.

The prevalence of malaria and the prevalence and patterns of malaria resistant mutations at baseline and at weeks 24 and 48 as well as the incidence of clinical malaria over the study period are calculated with 95% CI.

The primary safety analysis on the frequency and pattern of (S)AEs of the two groups at week 4 is performed using patient counts and percentages with 95% CIs, which are compared using Fisher's exact test. As a secondary objective, the safety of repeated courses of AM is compared with a single dose of PZQ, and with a single course of AM, using Poisson regression models, controlling for the administered number of doses.

ETHICS AND DISSEMINATION

Approvals

The study (Study Protocol V.1.5, 19 February 2020) is registered in Clinicaltrials.gov and carried out according to the principles stated in the Declaration of Helsinki, to all applicable regulations and according to the most recent GCP and Good Clinical Laboratory Practice guidelines. The study has been approved by the IRB of the ITM, Antwerp, Belgium (on 30 January 2019, Ref. 1269/18), by the EC of the Antwerp University Hospital, Belgium (on 21 January 2019, Ref. 19/02/005), and by the National Ethics Council for Research in Health (CNERS) in Senegal (on 24 April 2019, Ref. SEN19/08).

Data safety monitoring board

An independent DSMB, composed of a paediatrician and two schistosomiasis experts, has been established. The DSMB carefully monitors the quality of the data produced, and the safety and efficacy of the study drug, and advises on the (dis)continuation of the study should any major safety concern appear.

Consent

The school directors, teaching staff and community leaders of selected villages are informed about the study during a preparatory visit. After consent of the village leader, parents/guardians and their children are invited for a general information session and an individual consent process, during which information on schistosomiasis, the purpose, risks and benefits, and procedures of the trial is shared in local language and in a culturally sensitive and interactive way. A written informed consent from the parents or guardians and a verbal assent from the child are obtained by an experienced study nurse before procurement of the screening samples and later confirmed by the study physician during the baseline visit. All study personnel was trained in GCP. A separate section in the informed consent form is dedicated to the potential 50-year storage of the collected samples and their ancillary use, to which parents/guardians may or may not consent. The informed consent form used in the trial is provided in online supplemental additional file 3.

Withdrawal

Participation is voluntary and participants can withdraw from the study at any time for any reason without any consequences. However, follow-up for (S)AEs should proceed as planned until 4 weeks after the last drug administration.

In addition, participants are withdrawn from the study when the first drug administration is missed (eg, due to repeated vomiting), or in case of SAE. Participants withdrawing for medical reasons will be referred to adequate health facilities for further care if necessary. Unless consent is withdrawn, efforts will be made to schedule, for these participants, the week 24 and week 48 follow-up visits.

Randomised participants who miss one or more study visits remain in the study and are followed up as planned. Data collected up to the time of withdrawal will remain in the study database and be used for analysis whether or not the participant continues with the follow-up visits. No additional participants are recruited to replace those who have withdrawn. Participants who have been withdrawn from the study are offered a standard dose of PZQ after the final study visit.

Confidentiality

Personal and medical information from the trial is kept confidential, in line with the requirements of the European General Data Protection Regulation 2016/679, and with applicable requirements in Senegal. Access to all paper and electronic study files is restricted to authorised study staff. All personal data collected in the case report forms and the study database will be pseudonymised, by assigning a unique trial-specific code to each study participant.

Reporting

The study protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines. The SPIRIT protocol checklist is given in online supplemental additional file 4. The results of this trial will be presented at relevant national and international meetings and published in peer-reviewed journals regardless of outcome and in accordance with the Consolidated Standards of Reporting Trials statement and Standards for Reporting of Diagnostic Accuracy Studies2015 guidelines.

Dissemination

The participant level data set will be made available for access from third party researchers through a data repository as detailed in the data-sharing plan on Clinicaltrials.gov. The results will be disseminated in peer-reviewed journals and at international conferences.

TRIAL STATUS

This open-label, two-arm, individually randomised controlled trial has started screening in October 2019 and randomisation in November 2019. We envisage the sample collection period to be finished by the end of 2020, and sample processing and testing at ITM to start early 2021.

DISCUSSION

The SchistoSAM trial is the first proof of concept randomised controlled trial with an adequate power to evaluate the efficacy and safety of the antimalarial ACT AM as an alternative to PZQ for the treatment of schistosomiasis. Positive results will pave the way for future trials to evaluate AM in settings with different levels of malaria and schistosomiasis endemicity; in different treatment indications (eg, in adults); in second-line for PZQ resistant schistosomiasis; in first-line for malaria–schistosomiasis coinfected individuals; as a control strategy for schistosomiasis in malaria non-endemic areas; and/or as seasonal chemoprophylaxis for both conditions in co-endemic regions, where the dual activity of AM could favourably impact the cumulative individual and public health burden of both conditions. Our study also provides a unique and comprehensive validation of a range of top candidate diagnostic tools for monitoring therapeutic response. Hence, this trial is a key step towards the realisation of much needed alternative treatment strategies and monitoring tools for schistosomiasis control.

Author affiliations
[1]Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
[2]Department of Clinical Sciences, Institute of Tropical Medicine, Antwerpen, Belgium
[3]Department of Immunology, Cheikh Anta Diop University, Dakar, Senegal
[4]Institute for Health Research, Epidemiological Surveillance and Training (IRESSEF), Dakar, Senegal
[5]Department of Parasitology, Cheikh Anta Diop University, Dakar, Senegal

[6]Department of Health Sciences, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Contributors EB and KP conceptualised and designed the study. EB, KP, CR, IB, MM, ADH, BS, DA, CB, AT and YvH drafted the protocol. CR, IB, EB and KP drafted the manuscript. BTF, LP, IP, YvH, ADH, AT, BC and SM revised the manuscript. All authors read and approved the final version of the manuscript.

Funding This study is supported by the Flemish Ministry of Economy, Sciences and Innovation (EWI) which was granted in the framework of the Structural Research Funding of ITM (SOFI), grant number DIR/av/2018/90.

Disclaimer The funders had no role in study design, decision to publish the study protocol, or preparation of the manuscript.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer-reviewed.

ORCID iD

Isabel Brosius http://orcid.org/0000-0003-2227-8874

REFERENCES

1 Colley DG, Bustinduy AL, Secor WE, *et al*. Human schistosomiasis. *Lancet* 2014;383:2253–64.
2 WHO. *Global health estimates 2016: deaths by cause, age, sex, by country and by region, 2000-2016*, 2018.
3 GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the global burden of disease study 2016. *Lancet* 2017;390:1151–210.
4 GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet* 2017;390:1211–59.
5 Murray CJL, Vos T, Lozano R, *et al*. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the global burden of disease study 2010. *Lancet* 2012;380:2197–223.
6 McManus DP, Dunne DW, Sacko M, *et al*. Schistosomiasis. *Nat Rev Dis Primers* 2018;4:13.
7 King CH, Dickman K, Tisch DJ. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* 2005;365:1561–9.
8 Zwang J, Olliaro P. Efficacy and safety of praziquantel 40 mg/kg in preschool-aged and school-aged children: a meta-analysis. *Parasit Vectors* 2017;10:47.
9 Zwang J, Olliaro PL. Clinical efficacy and tolerability of praziquantel for intestinal and urinary schistosomiasis-a meta-analysis of comparative and non-comparative clinical trials. *PLoS Negl Trop Dis* 2014;8:e3286.
10 WHO preventive chemotherapy in human helminthiasis 2006.
11 Meurs L, Mbow M, Vereecken K, *et al*. Epidemiology of mixed Schistosoma mansoni and Schistosoma haematobium infections in northern Senegal. *Int J Parasitol* 2012;42:305–11.
12 Webster BL, Diaw OT, Seye MM, *et al*. Praziquantel treatment of school children from single and mixed infection foci of intestinal and urogenital schistosomiasis along the Senegal River Basin: monitoring treatment success and re-infection patterns. *Acta Trop* 2013;128:292–302.
13 Bergquist R, Utzinger J, Keiser J. Controlling schistosomiasis with praziquantel: how much longer without a viable alternative? *Infect Dis Poverty* 2017;6:74.
14 Cioli D, Pica-Mattoccia L, Basso A, *et al*. Schistosomiasis control: praziquantel forever? *Mol Biochem Parasitol* 2014;195:23–9.
15 Wu W, Wang W, Huang Y-X. New insight into praziquantel against various developmental stages of schistosomes. *Parasitol Res* 2011;109:1501–7.
16 Wang W, Wang L, Liang Y-S. Susceptibility or resistance of praziquantel in human schistosomiasis: a review. *Parasitol Res* 2012;111:1871–7.
17 Pinto-Almeida A, Mendes T, de Oliveira RN, *et al*. Morphological characteristics of Schistosoma mansoni PZQ-Resistant and -susceptible strains are different in presence of praziquantel. *Front Microbiol* 2016;7:594.
18 Vale N, Gouveia MJ, Rinaldi G, *et al*. Praziquantel for schistosomiasis: single-drug metabolism revisited, mode of action, and resistance. *Antimicrob Agents Chemother* 2017;61 doi:10.1128/AAC.02582-16
19 Geerts S, Gryseels B. Anthelmintic resistance in human helminths: a review. *Trop Med Int Health* 2001;6:915–21. doi:10.1046/j.1365-3156.2001.00774.x
20 Melman SD, Steinauer ML, Cunningham C, *et al*. Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of Schistosoma mansoni. *PLoS Negl Trop Dis* 2009;3:e504.
21 Siqueira LdaP, Fontes DAF, Aguilera CSB, *et al*. Schistosomiasis: drugs used and treatment strategies. *Acta Trop* 2017;176:179–87.
22 Liu R, Dong H-F, Guo Y, *et al*. Efficacy of praziquantel and artemisinin derivatives for the treatment and prevention of human schistosomiasis: a systematic review and meta-analysis. *Parasit Vectors* 2011;4:201.
23 Xiao S-hua. Mefloquine, a new type of compound against schistosomes and other helminthes in experimental studies. *Parasitol Res* 2013;112:3723–40.
24 Keiser J, Utzinger J. Antimalarials in the treatment of schistosomiasis. *Curr Pharm Des* 2012;18:3531–8.
25 Pérez del Villar L, Burguillo FJ, López-Abán J, *et al*. Systematic review and meta-analysis of artemisinin based therapies for the treatment and prevention of schistosomiasis. *PLoS One* 2012;7:e45867.
26 Elmorshedy H, Tanner M, Bergquist RN, *et al*. Prophylactic effect of artemether on human schistosomiasis mansoni among Egyptian children: a randomized controlled trial. *Acta Trop* 2016;158:52–8.
27 Keiser J, Chollet J, Xiao S-H, *et al*. Mefloquine-an aminoalcohol with promising antischistosomal properties in mice. *PLoS Negl Trop Dis* 2009;3:e350.
28 Abou-Shady OM, Mohammed SS, Attia SS, *et al*. Therapeutic effect of mefloquine on Schistosoma mansoni in experimental infection in mice. *J Parasit Dis* 2016;40:259–67.
29 Keiser J, N'Guessan NA, Adoubryn KD, *et al*. Efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, and praziquantel against Schistosoma haematobium: randomized, exploratory open-label trial. *Clin Infect Dis* 2010;50:1205–13.
30 Basra A, Mombo-Ngoma G, Melser MC, *et al*. Efficacy of mefloquine intermittent preventive treatment in pregnancy against Schistosoma haematobium infection in Gabon: a nested randomized controlled assessor-blinded clinical trial. *Clin Infect Dis* 2013;56:e68–75.
31 Cohee LM, Opondo C, Clarke SE, *et al*. Preventive malaria treatment among school-aged children in sub-Saharan Africa: a systematic review and meta-analyses. *Lancet Glob Health* 2020;8:e1499–511.
32 Utzinger J, Becker SL, van Lieshout L, *et al*. New diagnostic tools in schistosomiasis. *Clin Microbiol Infect* 2015;21:529–42.
33 de Vlas SJ, Gryseels B. Underestimation of Schistosoma mansoni prevalences. *Parasitol Today* 1992;8:274–7.
34 Knopp S, Becker SL, Ingram KJ, *et al*. Diagnosis and treatment of schistosomiasis in children in the era of intensified control. *Expert Rev Anti Infect Ther* 2013;11:1237–58.
35 Engels D, Sinzinkayo E, De Vlas SJ, *et al*. Intraspecimen fecal egg count variation in Schistosoma mansoni infection. *Am J Trop Med Hyg* 1997;57:571–7.
36 Verweij JJ, Stensvold CR. Molecular testing for clinical diagnosis and epidemiological investigations of intestinal parasitic infections. *Clin Microbiol Rev* 2014;27:371–418.
37 Meurs L, Brienen E, Mbow M, *et al*. Is PCR the next reference standard for the diagnosis of Schistosoma in stool? A comparison with microscopy in Senegal and Kenya. *PLoS Negl Trop Dis* 2015;9:e0003959.
38 ten Hove RJ, Verweij JJ, Vereecken K, *et al*. Multiplex real-time PCR for the detection and quantification of Schistosoma mansoni and

S. haematobium infection in stool samples collected in northern Senegal. *Trans R Soc Trop Med Hyg* 2008;102:179–85.
39 Vinkeles Melchers NVS, van Dam GJ, Shaproski D, *et al*. Diagnostic performance of Schistosoma real-time PCR in urine samples from Kenyan children infected with Schistosoma haematobium: day-to-day variation and follow-up after praziquantel treatment. *PLoS Negl Trop Dis* 2014;8:e2807.
40 RMD technical brochure-rapid test for qualitative detection of: bilharzia (schistosomiasis).
41 Greter H, Krauth SJ, Ngandolo BNR, *et al*. Validation of a point-of-care circulating cathodic antigen urine cassette test for Schistosoma mansoni diagnosis in the Sahel, and potential cross-reaction in pregnancy. *Am J Trop Med Hyg* 2016;94:361–4.
42 Armoo S, Cunningham LJ, Campbell SJ, *et al*. Detecting Schistosoma mansoni infections among pre-school-aged children in southern Ghana: a diagnostic comparison of urine-CCA, real-time PCR and Kato-Katz assays. *BMC Infect Dis* 2020;20:301.
43 Peralta JM, Cavalcanti MG. Is POC-CCA a truly reliable test for schistosomiasis diagnosis in low endemic areas? the trace results controversy. *PLoS Negl Trop Dis* 2018;12:e0006813.
44 Adriko M, Standley CJ, Tinkitina B, *et al*. Evaluation of circulating cathodic antigen (CCA) urine-cassette assay as a survey tool for Schistosoma mansoni in different transmission settings within Bugiri district, Uganda. *Acta Trop* 2014;136:50–7.
45 Lamberton PHL, Kabatereine NB, Oguttu DW, *et al*. Sensitivity and specificity of multiple Kato-Katz thick smears and a circulating cathodic antigen test for Schistosoma mansoni diagnosis pre- and post-repeated-praziquantel treatment. *PLoS Negl Trop Dis* 2014;8:e3139.
46 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;6:63.
47 Colley DG, Binder S, Campbell C, *et al*. A five-country evaluation of a point-of-care circulating cathodic antigen urine assay for the prevalence of Schistosoma mansoni. *Am J Trop Med Hyg* 2013;88:426–32.
48 Corstjens PLAM, van Lieshout L, Zuiderwijk M, *et al*. Up-converting phosphor technology-based lateral flow assay for detection of Schistosoma circulating anodic antigen in serum. *J Clin Microbiol* 2008;46:171–6.
49 Polman K, Diakhate MM, Engels D, *et al*. Specificity of circulating antigen detection for schistosomiasis mansoni in Senegal and Burundi. *Trop Med Int Health* 2000;5:534–7.
50 van Dam GJ, Bogitsh BJ, van Zeyl RJ, *et al*. Schistosoma mansoni: in vitro and in vivo excretion of CAA and CCA by developing schistosomula and adult worms. *J Parasitol* 1996;82:557–64.
51 van Dam GJ, de Dood CJ, Lewis M, *et al*. A robust dry reagent lateral flow assay for diagnosis of active schistosomiasis by detection of Schistosoma circulating anodic antigen. *Exp Parasitol* 2013;135:274–82.
52 Knopp S, Corstjens PLAM, Koukounari A, *et al*. Sensitivity and specificity of a urine circulating anodic antigen test for the diagnosis of Schistosoma haematobium in low endemic settings. *PLoS Negl Trop Dis* 2015;9:e0003752.
53 Corstjens PLAM, Nyakundi RK, de Dood CJ, *et al*. Improved sensitivity of the urine CAA lateral-flow assay for diagnosing active Schistosoma infections by using larger sample volumes. *Parasit Vectors* 2015;8:241.
54 Hatz CF. The use of ultrasound in schistosomiasis. *Adv Parasitol* 2001;48:225–84.
55 Barda B, Coulibaly JT, Hatz C, *et al*. Ultrasonographic evaluation of urinary tract morbidity in school-aged and preschool-aged children infected with Schistosoma haematobium and its evolution after praziquantel treatment: a randomized controlled trial. *PLoS Negl Trop Dis* 2017;11:e0005400.
56 Webster JP, Koukounari A, Lamberton PHL, *et al*. Evaluation and application of potential schistosome-associated morbidity markers within large-scale mass chemotherapy programmes. *Parasitology* 2009;136:1789–99.
57 Ochodo EA, Gopalakrishna G, Spek B, *et al*. Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas. *Cochrane Database Syst Rev* 2015:CD009579.
58 Emukah E, Gutman J, Eguagie J, *et al*. Urine heme dipsticks are useful in monitoring the impact of praziquantel treatment on Schistosoma haematobium in sentinel communities of delta state, Nigeria. *Acta Trop* 2012;122:126–31.
59 Bustinduy AL, Sousa-Figueiredo JC, Adriko M, *et al*. Fecal occult blood and fecal calprotectin as point-of-care markers of intestinal morbidity in Ugandan children with Schistosoma mansoni infection. *PLoS Negl Trop Dis* 2013;7:e2542.
60 Seck MC, Thwing J, Fall FB, *et al*. Malaria prevalence, prevention and treatment seeking practices among nomadic pastoralists in northern Senegal. *Malar J* 2017;16:413.
61 WHO. *Basic laboratory methods in medical parasitology*. Geneva: World Health Organization, 1991.
62 Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo* 1972;14:397–400.
63 Casacuberta-Partal M, Hoekstra PT, Kornelis D, *et al*. An innovative and user-friendly scoring system for standardised quantitative interpretation of the urine-based point-of-care strip test (POC-CCA) for the diagnosis of intestinal schistosomiasis: a proof-of-concept study. *Acta Trop* 2019;199:105150.
64 Corstjens PLAM, De Dood CJ, Kornelis D, *et al*. Tools for diagnosis, monitoring and screening of Schistosoma infections utilizing lateral-flow based assays and upconverting phosphor labels. *Parasitology* 2014;141:1841–55.
65 van Grootveld R, van Dam GJ, de Dood C, *et al*. Improved diagnosis of active Schistosoma infection in travellers and migrants using the ultra-sensitive in-house lateral flow test for detection of circulating anodic antigen (CAA) in serum. *Eur J Clin Microbiol Infect Dis* 2018;37:1709–16.
66 Pillay P, Taylor M, Zulu SG, *et al*. Real-Time polymerase chain reaction for detection of Schistosoma DNA in small-volume urine samples reflects focal distribution of urogenital schistosomiasis in primary school girls in KwaZulu natal, South Africa. *Am J Trop Med Hyg* 2014;90:546–52.
67 Obeng BB, Aryeetey YA, de Dood CJ, *et al*. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. *Ann Trop Med Parasitol* 2008;102:625–33.
68 Richter J, Campange G, Hatz G. *Ultrasound in schistosomiasis. A practical guide to the standardized use of ultrasonography for the assessment of Schistosomiasis-related morbidity*. World Health Organization, 2016.
69 WHO haemoglobin concentrations for the diagnosis of anaemia and assessment of severity 2011.
70 Hofmann N, Mwingira F, Shekalaghe S, *et al*. Ultra-sensitive detection of Plasmodium falciparum by amplification of multi-copy subtelomeric targets. *PLoS Med* 2015;12:e1001788.
71 Oyebola KM, Aina OO, Idowu ET, *et al*. A barcode of multilocus nuclear DNA identifies genetic relatedness in pre- and post-Artemether/Lumefantrine treated Plasmodium falciparum in Nigeria. *BMC Infect Dis* 2018;18:392.
72 Witkowski B, Duru V, Khim N, *et al*. A surrogate marker of piperaquine-resistant Plasmodium falciparum malaria: a phenotype-genotype association study. *Lancet Infect Dis* 2017;17:174–83.
73 Ariey F, Witkowski B, Amaratunga C, *et al*. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. *Nature* 2014;505:50–5.
74 Vachot-Ganée L, Khim N, Iannello A, *et al*. A novel field-based molecular assay to detect validated artemisinin-resistant K13 mutants. *Malar J* 2018;17:175.
75 Agency EM ICH topic E 9 statistical principles for clinical trials step 5. note for guidance on statistical principles for clinical trials (CPMP/ICH/363/96) 1998.