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The diagnostic value of focused assessment with sonography for urinary schistosomiasis (FASUS) for morbidity detection in endemic regions

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ABBREVIATIONS

AM	Ante meridiem
AIDS	Acquired immune deficiency syndrome
AWA	Adult worm antigen
BMI	Body mass index
CAA	Circulating anodic antigen
CCA	Circulating cathodic antigen
CERMEL	Centre de Recherches Médicales de Lambaréné
CRF	Case report form
CT	Computerised tomography
DEET	Diethyltoluamide
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FASE	Focused assessment with sonography for echinococcosis
FASH	Focused assessment with sonography for HIV-associated tuberculosis
FAST	Focused assessment with sonography for trauma
FASUS	Focused assessment with sonography for urinary schistosomiasis
FGS	Female genital schistosomiasis
GDP	Gross domestic product
HAS	Albert Schweitzer Hospital (Hôpital Albert Schweitzer)
HIV	Human immune-deficiency virus
ICH-GCP	International Conference of Harmonisation of Good Clinical Practice
IgE	Immunoglobulin E
ITS	Internal transcribed spacer
IQR	Inter quartile range
MDA	Mass drug administration
MGS	Male genital schistosomiasis
MHZ	Megahertz
MRI	Magnetic resonance imaging

M1	Follow up one month after treatment
M3	Follow up three months after treatment
NTD	Neglected tropical diseases
PCR	Polymerase chain reaction
PDF	Portable Document Format
phHV	Phocine herpesvirus
PM	Post meridiem
POCUS	Point of care Ultrasound
PSAC	Pre-school aged children
PZQ	Praziquantel
RNA	Ribonucleic acid
rpm	Rounds per minute
SAC	School aged children
SEA	Soluble egg antigen
SmCTF	Cercarial transformation fluid
SOP	Standard operating procedure
Th2	T helper cell 2
UGS	Urogenital schistosomiasis
US	Ultrasound
USB	Universal serial bus
UT	Urinary tract
UTI	Urinary tract infection
WHO	World health organization

1 Introduction

1.1 Schistosomiasis

Schistosomiasis belongs to the neglected tropical diseases (NTD) and is one of the most widespread parasitic diseases in the world accounting for a substantial burden of morbidity in endemic countries. In 2018 209 million people were in need of treatment for schistosomiasis [1].

1.1.1 Microbiology

Schistosomiasis is caused by parasitic trematode flatworms of the genus *schistosoma* endemic in tropical and subtropical regions [2]. The seven to twenty-two millimetres long worm was first described in 1852 by Theodor Bilharz [3]. Therefore the disease is also known as “la bilharziose” in French speaking countries.

There are six main species that cause infection in humans. *Schistosoma haematobium* is causing urogenital schistosomiasis (UGS). The other five species are *S. mansoni, S. mekongi, S. japonicum, S. intercalatum* and *S. guineensis*. All of them mainly cause intestinal schistosomiasis with *S. mansoni* and *S. japonicum* being the most common ones [2, 4].

Humans are the primary host for *S. mansoni, S. japonicum* and *S. haematobium. S. intercalatum* and *S. mekongi* are primarily zoonotic and can also be found in many mammalian hosts, complicating their elimination [5].

1.1.2 Life cycle

Schistosoma infection is acquired during swimming or wading in slow running or standing fresh water populated by its vector, small freshwater snails.

Schistosoma species show a specificity regarding the species of their intermediate snail host. Hence the geographical distribution of the snails (which mainly depends on environmental factors) is an important factor that indicates the spread of *schistosoma* species (described in 1.1.4) [6, 7]. While *S. haematobium* and *S. intercalatum* both use *Bulinus* species as intermediate

host, *S. mansoni* prefers *Biomphalaria* species. *S. japonicum* is found in *Oncomelania* species and *S. mekongi* in *Neotricula* [2, 8-10].

Schistosoma eggs are released into the water via urine (*S. haematobium*) or stool (all others) of an infected individual. Excreted eggs have a viability of up to seven days [3]. The eggs hatch and release miracidiae that find and penetrate their specific intermediate snail host.

Inside the snail, the miracidiae multiply asexually and within four to six weeks first become multicellular sporocysts and then cercarial larvae, the infectious form of the parasite, which is able to penetrate the human skin. When released by the snail the larvae can survive about 72 hours while searching for a definite human host [3]. Human skin is mostly penetrated in the foot area while wading through freshwater. After penetration the larvae discard their tails and become schistosomulae inside the human organism.

Feeding from their host's blood, the parasites migrate via the lung into the portal vein where they develop into adult worms and mate with a life long partner of the opposite sex. Within four to six weeks the couples migrate as a unit to their definite destination, the venous plexus of different organs.

Located in the venules the female worms produce hundreds or thousands of eggs per day. A worm's average lifetime are three to five years but may also reach up to 30 years [11]. Consequently one couple can produce around 600 billion new schistosomes over their lifetime.

Some eggs make their way through the tissue and are excreted via stool or urine of the respective individual, which completes the parasitic life cycle [2, 3].

Other migrating eggs get stuck on their way and cause an immune reaction, which is mainly responsible for the pathology and symptoms of schistosomiasis.

1.1.3 Pathogenesis and clinical features

Final habitats to the mating worms are the venous plexi of different human organs. *S. haematobium* migrates to the venous plexus of urinary bladder, ureter and genital region. While the other species like *S. japonicum*, *S. mansoni, S. intercalatum*, *S. mekongi* and *S. guineensis* prefer the mesenteric venules of the small and large gut [12]. In some rare cases the worms might

also be found in the lungs, the liver, spleen or even brain, spinal cord or skin [13].

In order to migrate through the human tissue and reach the lumen of the ureter/bladder or bowel the eggs excrete a proteolytic enzyme that triggers an eosinophilic inflammatory and granulomatous reaction [14].

Under chronic infection macrophages, eosinophiles, lymphocytes and giant cells form granulomas. Thereby the body is able to destroy or at least encapsulate the eggs, but in the long run it will lead to fibrosis of the affected tissue. Granulomas and consecutive organ fibrosis play a major role in schistosomiasis-related morbidity [15].

In contrary to the eggs, the adult worms are less immunogenic and cause no or little immune reaction [16].

There are acute as well as chronic manifestations of a schistosomal infection. In many cases the disease is defeated by the immune system without any therapy and early pathological changes are reversible. However, untreated chronic infection can lead to severe organ dysfunction and secondary diseases [17].

1.1.3.1 Acute schistosomiasis infection

Swimmer's itch

Usually the parasites' skin penetration is asymptomatic. But in some cases, a cercarial dermatitis develops in the penetration area. It presents as a transient pruritic rash that may develop within 12 hours after parasite contact [18]. Spontaneous regression can be observed within one day or maximum three weeks. It is suggested that swimmers itch mainly occurs when humans get infected with zoonotic *schistosoma* species [19].

Katayama fever/syndrome

Acute *schistosoma* infection is also rarely symptomatic; in case it is symptomatic it is called "Katayama fever" or "Katayama syndrome". It might occur about three to six weeks after primary infection or heavy reinfection with *S. mansoni*, *S. japonicum* and rarely also *S. haematobium* and is thereby mainly reported in immune-naive travellers. It is triggered by a hypersensitive

immune reaction to parasitic and egg antigens [20]. It is a matter of discussion whether children in endemic regions are already desensitized *in utero* and are therefore rarely affected by symptomatic acute infection. Katayama fever may also simply be an under diagnosed manifestation of schistosomiasis in endemic populations [20].

The broad variation of symptoms makes Katayama syndrome hard to diagnose. Patients may present with fever, urticarial rash and eosinophilia as well as myalgia and headache. Abdominal tenderness due to enlargement of spleen and liver has also been observed in many cases [18, 21]. Pulmonary symptoms are also common and manifest as bronchospasm, dry cough, wheezing and thoracic pain. The accompanying radiologic changes present as thickening of bronchial walls as well as beaded micronodulation [22].

Most patients recover spontaneously within two to ten weeks. However, some individuals develop a more severe form with dyspnea, generalized rash and weight loss due to gastrointestinal symptoms such as diarrhea, nausea and vomiting [23]. The response to schistosomiasis specific treatment is usually well but sometimes additional treatment with steroids might be required [20].

Neuroschistosomiasis

Acute neuroschistosomiasis is defined as *schistosoma* involvement in spine cord or brain (central nervous system) and is the most dangerous and dreaded manifestation of *schistosoma* infection. It usually begins three weeks after general manifestation but can occur in any infection stage. Although much less common than the systemic acute infection or chronic intestinal or urogenital infection, neuroschistosomiasis probably is an under recognized manifestation of this disease. It most often occurs with *S. mansoni* and *S. japonicum* but also *S. haematobium* infection. *S. mansoni* and *S. haematobium* mostly cause transverse myelitis as a neurological manifestation while *S. japonicum* can cause encephalitic disease [20]. The outcome strongly depends on an early diagnosis and treatment [24].

1.1.3.2 Chronic infection

Urogenital schistosomiasis

Urogenital schistosomiasis (UGS) is the typical manifestation of *S. haematobium* infection.

In rare cases urological or genital symptoms can also be induced by another species' eggs (for example *S. mansoni)* embolized into the perivesicular or genital plexus [25, 26].

The migrating eggs cause granulomatous inflammation in the bladder wall, the ureters and the genital tract. Fibrosis, ulcerations and submucosal granulomas of bladder, genital tract and ureteral walls are the consequence.

The most common clinical symptom of UGS is hematuria. Intensity varies from micro-hematuria to severe macro-hematuria with red or brownish urine colour. Symptoms of the genital tract are as described below.

In long-term infected individuals fibrosis can result in an occlusion of the ureters and consecutive hydronephrosis. While hydronephrosis is reversible in most cases, sometimes even spontaneously, it can also lead to renal failure in more severe and untreated cases [27].

Some eggs might also lead to a calcification of the bladder wall and the ureters which can be observed in X-ray or ultrasound [3].

Chronic *S. haematobium* infection is also associated with an increased risk for squamous cell carcinoma of the blader [3, 28]. The share of squamous cell carcinoma in the over all number of bladder carcinomas is around five percent in western countries but up to 75% in countries where *S. haematobium* is endemic [29-31].

Although it was shown that urogenital symptoms are often reversible (81%) after treatment, morbidity is still high due to unsatisfying control programs in many endemic areas. It is controversial if there is a specific mortality due to *S. haematobium* infection. While some data suggest that especially older individuals have an increased specific mortality due to renal failure, other studies do not support these results [3, 32].

Female genital schistosomiasis

Although the egg burden in the male as well as female genital tract has been shown to be rather low compared to urinary bladder and ureters the consequences of genital infection may be severe [33].

When worms migrate from the vesical venous plexus via the recto-vesical plexus into the genital region, *S. haematobium* (and less often also the other subspecies) can cause female genital schistosomiasis (FGS). It mainly affects vagina, cervix and fallopian tubes. In UGS endemic areas, FGS is estimated to affect 30-50% of women [34]. It may present with vaginal bleeding and discharge, itching and pain or bleeding during sexual intercourse. Women's' fertility can be impaired when the upper reproductive tract is affected. Granulomatous inflammation in fallopian tubes can cause fibrosis and occlusion with the result of a high risk of ectopic pregnancy and infertility. Involvement of uterus or placenta can lead to spontaneous abortion in pregnant women [25, 35].

Additionally a study from Ghana demonstrated that women infected with *S. haematobium* were more likely to have preterm deliveries with an even lower birth weight compared to preterm deliveries of the control group [36].

Infection of the lower reproductive tract manifests with itchy patches at vulva and vagina. Mucosal lesions of the vagina present as "*sandy patches*" or "*rubbery papules*" that can be identified in colposcopic examination [37, 38].

Due to the epithelial damage within mucosal lesions of the lower reproductive tract, the risk of acquisition and transmission of HIV (Human immune-deficiency virus) infection during sexual intercourse is increased [39-41]. The same applies for vertical transmission from an infected mother to her baby during birth. Additionally, *schistosomae* have immune-modulating effects that promote infection as well as progression of HIV infection in schistosoma co-infected individuals [39-41].

Male genital schistosomiasis

The worms or eggs reach the male genital tract by migration through a common anastomosis between the periumbilical veins and the spermic venous plexus.

Male genital schistosomiasis (MGS) is most common with *S. haematobium* but has also been reported after *S. mansoni* and *S. intercalatum* infection [42].
The most common clinical symptom of genital schistosomiasis in men is hematospermia, but symptoms like testicular or prostate swelling due to epididymitis or prostatitis have also been observed [43, 44]. Reasons for heamatospermia seem to be inflammation in the seminal vesicles as well as sclerosing endophlebitis in the spermatic cord [45]. Sometimes eggs can be found in the ejaculate. Just as in women lesions and fibrosis of the prostate and the funiculus spermaticus can lead to infertility.
There is also a case report about *S. haematobium* associated prostate adenocarcinoma [46].
In contrast to FGS epithelial lesions do not affect the male outer reproductive tract. Therefore, male patients with schistosomiasis are not at increased risk for HIV infection due to a mucosal barrier dysfunction. However, the immunosuppression induced by the parasites still increases the risk of HIV progression in patients with MGS substantially [39].

Intestinal and hepatosplenic schistosomiasis

All schistosoma species other than *S. haematobium* primarily cause chronic inflammation in liver, spleen and bowel.
Patients suffering from intestinal schistosomiasis mostly present with unclear abdominal pain and recurrent or chronic diarrhea sometimes accompanied by bloody stools due to intestinal bleeding [27]. The chronic inflammation of the bowel caused by the eggs can lead to blood and protein loss, intestinal polyposis and ulceration [12]. The association with an increased risk of colon carcinoma is controversial. In severe cases colonic and rectal stenosis have been reported and even a case of colon perforation due to obstruction as a consequence of *S. mansoni* infection [8, 27].
In the liver veins the eggs can trigger periportal fibrosis (Symmers' pipestem fibrosis) and consecutive portal hypertension [8, 27]. After some time this can lead to esophageal varicosis, which pose a high risk of bleeding.

In contrast to liver cirrhosis of other etiologies, the synthetic functions of the liver are usually unimpaired until very late stages [47].
However, progressive cirrhosis of the liver is associated with an increased risk of hepatocellular carcinoma [8].
Calcified eggs in the gall bladder can trigger the formation of gallstones [48].

Glomerular disease

Besides renal pathology caused by fibrosis of the lower urinary tract due to *S. haematobium* infection as described in 1.1.3.2, schistosomiasis can also damage the kidney in other ways. Glomerular disease is mainly associated with chronic *S. mansoni* infection. The infection can cause immune-mediated glomerulonephritis as well as tubular injury due to oxidant stress [49, 50].
In most cases schistosomiasis-associated glomerular disease is subclinical or resolves spontaneously, but it might also persists or even progresses to nephrotic syndrome [51] causing proteinuria, hypoproteinemia and edema [52].
While in western countries membranous glomerulonephritis is the main cause of nephrotic syndrome in adults, in many African countries infectious diseases like HIV and schistosomiasis play a major role in the pathology of this syndrome [53, 54].

Pulmonary schistosomiasis

The chronic pulmonary manifestation of schistosomiasis is less common than acute pulmonary symptoms (Katayama fever). Granulomatous infection triggered by *schistosoma* eggs leads to pulmonary granulomatous schistosomiasis or pulmonary hypertension sometimes resolving in cor pulmonale as well as arterio-venous fistulae [55].

Anemia

All types of *schistosoma* infection have in common that they are often associated with lower hemoglobin levels and anemia and therefore can lead to general fatigue [56]. Since other factors like co-infections and malnutrition are

often present simultaneously, the isolated effect of schistosomiasis is hard to determine.

1.1.4 Epidemiology of schistosomiasis

Schistosomiasis is mainly endemic in rural areas of the tropics and subtropics. Many of these regions can be classified as resource-limited areas. Disease-control is thus made even more difficult by issues such as lack of proper sanitary facilities, clean water sources and a working health care system with sufficient schistosomiasis control programs.

The six different species infecting humans are found in different regions, which are sometimes overlapping. *S. haematobium* is endemic in many regions of the African continent, mainly sub-Saharan Africa and parts of the Middle East. *S. mansoni* is also found in many regions of Africa, the Middle East, South America and the Caribbean. *S. mekongi* is more of regional importance and only endemic in the Mekong river basin of Southeast Asia. *S. japonicum* on the other hand is widely spread over China and the Philippines. *S. intercalatum* is also found in central and west Africa, while *S. guineensis* has only been found in the rainforest areas of central Africa [2, 4].

Based on the report "*Schistosomiasis and soil- transmitted helminthiases: numbers of people treated in 2018*" by the World Health Organization (WHO) [1] it was estimated that in 2018 a total of 229.2 million individuals in 52 countries were in need of anti-schistosomal treatment. Of these 54.2% (124.4 million) were estimated to be school-aged children (SAC).

The same year 95.3 million people actually received therapy for schistosomiasis of which 76.2 million were SAC. This equals a global coverage of 41.5%.

The vast majority (89%) of people in need of treatment are living on the African continent.

1.1.5 Diagnosis of urogenital schistosomiasis

Diagnostic approaches to detect *S. haematobium* infection in humans can be classified into four different categories [57]: *"(i) direct parasitological diagnosis; (ii) immunological diagnosis; (iii) DNA*" (deoxyribonucleic acid) "*and RNA*"

(ribonucleic acid) "*detection; and (iv) use of cytokines, metabolites, and other schistosome molecules as biomarkers*." There is no test with 100 percent sensitivity [58]. Especially in patients with low worm burden, e.g. returning travellers, a combination of several screening tools is necessary to reach a satisfactory sensitivity [59].

1.1.5.1 Laboratory methods

Urine microscopy

Microscopy of urine samples to determine egg excretion is the gold standard in *S. haematobium* diagnosis.

The main advantages of this method are its comparably low costs and labour intensity. Sensitivity is high for heavy infection but may be inferior to other methods when it comes to early and light infection. Despite this it is the most widely used technique to diagnose UGS. Especially in endemic regions where patients are expected to bear a high worm burden and resources are limited it plays a major role as community based screening tool [57, 60].

A urine sample that has been collected from the individual in question is filtrated though a filter membrane which is then examined under the microscope in order to identify eggs that have been excreted via urine. By using urine filtration or centrifugation prior to the microscopic analysis sensitivity can be increased [61]. The *Schistosoma* species can be determined based on egg shape.

The approximate determination of worm burden based on the egg count is possible but not very accurate. Excretion depends on several factors such as e.g. daytime and host age. Egg excretion seems to be the highest between 10 am (ante meridiem) and 2 pm (post meridiem), therefore collection at this time of day may increase the sensitivity of urine microscopy [62]. For *S. haematobium* an egg burden of over 50 eggs / 10 ml is classified as heavy infection.

By collecting urine samples on several consecutive days, the drawbacks of low sensitivity for light infection as well as daily fluctuation in egg excretion can be reduced.

The technical details of urine microscopy are described in the methods section.

Urine dipstick analysis

Urine analysis with urinary dipstick is also widely used in endemic areas. Hematuria and proteinuria have been shown to be associated with *S. haematobium* infection. Although they can also appear as symptoms of various other diseases urinary dipstick combined with a questionnaire focusing on other UGS symptoms can be used as a screening method in endemic regions [57].

Polymerase chain reaction

Urine, serum, saliva, semen or vaginal lavage fluids can be analysed via PCR for egg DNA, circulating cell-free DNA and circulating microRNAs of *schistosomes*.

DNA extraction and Polymerase chain reaction (PCR) of urine samples are described in detail in the methods section.

The genetic targets for *S. haematobium* egg DNA that are available at the moment are draI repeats, cox 1, internal transcribed spacer DNA (ITS) and ITS2. Sensitivity for urine analysis is slightly higher than in urine microscopy [63, 64] and it can be used as a qualitative as well as quantitative tool to detect worm burden and distinguish between species [65]. However, depending on the specimen that is used, PCR diagnosis can be affected by the same problems as microscopy (for example fluctuation of egg excretion in urine).

Due to high material and equipment costs as well as the need for highly trained staff, PCR is mainly used in resource-rich and scientific contexts and not as a screening or diagnostic tool in endemic areas.

Additional techniques such as Loop-mediated Isothermal Amplification (LAMP) have been shown to be able to improve PCR sensitivity [66].

Immunologic testing

Immunologic tests can detect anti-schistosomal antibodies or schistosomal antigens in patients' serum, sputum or urine.

They are able to determine schistosomiasis six to twelve weeks after infection [67]. Consequently acute infection will not be detected but both antigen as well

as antibody detection methods will usually be positive prior to microscopic diagnosis.

Due to their high costs and labour intensity immunological tests are most relevant in resource-rich settings and used for patients with light infections, which are not detectable by parasitological methods. Another disadvantage is the cross reactivity of the different *schistosoma* species as well as with other helminthes [68].

Antibody detection has a high sensitivity in diagnosing *S. haematobium* infection. However, the titers do not correlate with the parasite burden and persist in the blood for month or even years. Therefore it is not possible to distinguish an active from a prior infection and the test cannot be used for follow-up controls or in endemic regions [69].

Furthermore, it can't determine intensity of infection.

For *S. haematobium* antibodies against antigens like adult worm antigens (AWA) or cercarial transformation fluid (SmCTF) can be detected via ELISA (Enzyme-linked immunosorbent assay) or immunoelectrotransfer blot [57].

In contrast to the antibodies, antigen titers do correlate with clinical severity as well as intensity of infection. Five to ten days after elimination of *S. haematobium* via treatment antigens will no longer be detectable [70]. Commonly targeted antigens are AWA, soluble egg antigen (SEA), circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) in urine, blood or sputum. CCA and CAA can both be detected in urine or blood around four weeks after infection [71]. Sensitivity is highly dependent on the antigen used and ranges between 65% with soluble worm antigen (SWA) and 100% with microsomal *S. haematobium* AWA [68]. Although there are "*Circulating Cathodic Antigen point-of-care-test (POC-CCA)*" available, their sensitivity in rapid diagnosis in the field have been found lower than urine microscopy [72].

Antigen detection in urine also shares the disadvantage of dependency on day-to-day fluctuation in egg excretion [57].

Metabolic products and cytokines

Host cytokines and *schistosome* metabolites as markers for *schistosoma* infection have been assessed but are not very specific and consequently have a poor diagnostic value when used alone. Different methods have been used for detection, for example mass spectrometry [57].

1.1.5.2 Imaging methods

In contrast to laboratory tests, imaging methods are not commonly used as a tool to diagnose *S. haematobium* infection but to identify its morbidity and related sequelae. Pathologic changes such as hydronephrosis due to fibrosis of the ureter, bladder wall thickening and calcification can be determined.

X-ray and pyelography

Chest x-ray is mainly relevant in the diagnosis of pulmonary manifestations. Nodules and enlargement of the lung arteries can be seen as a consequence of pulmonary hypertension [57, 62, 73, 74].

Plain abdominal X-ray may be used to detect bladder wall calcification caused by *S. haematobium* infection. Due to high radiation it is not used routinely though.

Pyelography has also been used as a diagnostic tool for *S. haematobium* related hydronephrosis but because of its high invasiveness it is no longer of importance. Additionally it is not superior to the much less invasive ultrasonography [75].

Cystoscopy

Cystoscopy is used to determine bladder lesions in *S. haematobium* infected patients with consecutive egg detection in biopsy material. Because of its high invasiveness, however, it is exclusively used if less invasive diagnostic methods do not deliver sufficient information for further management or for scientific purposes [27, 76].

Ultrasound

Ultrasound (US) has been used in the diagnosis of schistosomiasis since 1987 [77]. Several studies could prove that ultrasound can provide diagnostic data comparable to cystoscopy for bladder lesions or intravenous pyelography for renal pathologies and thus is a valid and much less invasive tool in the detection of schistosomiasis induced pathologies [75, 78, 79]. US can be used as a low-risk diagnostic tool in detection of advanced schistosomiasis morbidity as well as a tool to monitor treatment success for morbidity control.

In 1991 the WHO assigned a group of experts to define a standardized ultrasound protocol for morbidity detection in schistosomiasis in order to make the data obtained all over the world comparable. In 1996 the protocol was reviewed in Niamey, Niger and then published as "*Niamey protocol*" [80].

A recent review investigated the Niamey protocol's "*Acceptance and evolution over 14 years*" and showed a good overall acceptance of the protocol after its publication. Between 2012 and 2014 it was applied in 75% of studies using sonography in *S. haematobium* infection [77].

Computerized tomography and magnetic resonance imaging

Computerized tomography (CT) and magnetic resonance imaging (MRI) are mainly relevant to detect cerebral or spinal foci when neuroschistosomiasis is suspected [62].

1.1.6 Treatment of schistosomiasis

Individual treatment of chronic infection

The treatment of schistosomiasis initially comprised drugs like antimonials (intravenous application), hycanthone and lucanthone (intramuscular administration). Soon these drugs were ruled out because of their impractical administration form as well as the severe side effects. Newer oral drugs like niridazole, oxamniquine and metrifonate were used alternatively. But just as their predecessors niridazole and oxamniquine they had major side effects. Only metrifonate showed good efficacy with only mild adverse drug effects. The main disadvantage of this drug is the need for multiple administrations, which

makes it unsuitable for widespread use [81]. In 1982 praziquantel (PZQ) was introduced to the market, an oral broad-spectrum anti-helminthic drug with high effectiveness and a low side effect profile. Studies showed an equal or even superior effectiveness of PZQ against all *schistosoma* species compared to other drugs [82]. For *S. haematobium* PZQ is most effective in a single dose of 40 mg/kg bodyweight four to six weeks after infection when worms are fully matured. PZQ has no effect on the parasites larval form [83, 84]. It leads to a contraction of the *schistosoma* due to a change of the permeability of its cell membrane and has been safely used for three decades now. It is considered safe for pregnant (after the first trimester [85]) and lactating women [83]. Although the dosage form in pills of about 1 cm of size is not ideal when treating younger children, PZQ is also found to be safe for pre-school age children (PSAC) [86]. It is possible and effective to crush the tablets and administer them in a medium like for example yoghurt [83].

Reported side effects are dizziness, abdominal cramps, nausea and vomiting but usually stay mild [87]. A study from Kenya showed that two to three months after a 40 mg/kg bodyweight PZQ dose, 84% of *S. haematobium* infected patients were cured and in non-cured individuals the parasite burden was reduced by over 90% [88]. Follow-up microscopy to determine egg count should not be performed earlier than six weeks after the first administration. If six to twelve weeks after treatment egg excretion persists, retreatment is recommended according to some literature [89, 90]. The resurgence of urinary tract pathologies may take 12 to 18 month, though [17].

Control programs and prevention

Although PZQ is a potent and well-tolerated agent for the treatment of schistosomiasis, other challenges have to be faced when treating large numbers of patients in endemic regions. The main problem is constant reinfection of individuals due to poor sanitary facilities and the lack of clean water sources. Since cure rates are still low the main focus of schistosomiasis treatment in endemic areas has been set on morbidity control [17].

The most widely and most successfully used method in schistosomiasis control has been mass drug administration (MDA) of PZQ. The WHO recommends annual preventive treatment in endemic countries for people at risk of infection, regardless of the individual infectious status, to aim for morbidity control and prevention of severe secondary illness due to chronic infection [91]. If applied regularly over a longer time span, MDA can reach a majority of people at risk and improve short-term as well as long-term health of the target population. Additionally, it expedites the development of immunity against *schistosoma* by enhancing the exposure of *schistosoma* antigens and therefore the development of specific antibodies. A strong *schistosoma*-triggered Th2 response and the consecutive production of IgE (Immunoglobulin E) antibodies against the adult worm seem to be a protective factor against reinfection after treatment [92-94]. Under natural circumstances this would only occur after several years of exposure.

It has been shown that multiple re-treatments reduce reinfection rates even further [95].

A study from 1992 demonstrated that yearly treatment with PZQ reduces severity of *S. haematobium* infection as well as morbidity detected by sonography [96]. Another study in Kenya showed that children who underwent annual treatment for 5 years in a row averagely showed the same prevalence of *S. haematobium* infection at reassessment after 7 to 13 years, but significantly less severe urinary tract pathologies by ultrasound [97]. This suggests that even with reinfection, MDA can reduce the long-term development of severe pathologies of the urinary tract.

The effect of chemotherapy with PZQ on more subtle pathologies like anemia, growth and nutrition state are controversial but seem to be more dependent on the treatment of intestinal helminthes (a very common co-infection of schistosomiasis, especially in children) as well as iron supplementation [17].

Since the "*World Health Assembly Resolution 65.19*" from 2012, the WHO advises countries which have successfully applied MDA to aim beyond morbidity control towards schistosomiasis elimination [85]. Japan for example states to have successfully eradicated *S. japonicum* infection since 1996 [98].

The eradication strategy, additionally to treatment of infected individuals, consisted of replacing susceptible animal reservoirs such as cows, draining of wetlands and cementing of ditches in order to control the vector snail population. Sodium pentachlorophenate was used as another snail eradication tool and sprayed profusely [99].

Molluscicides have been used widely in order to control the vector population before PZQ was available for mass drug administration. Due to rapid repopulation, the effects of solitary use in highly endemic regions have been limited though [100, 101]. Today molluscicides are mainly used additionally in countries that aim for elimination of schistosomiasis. Means of choice is niclosamine. Administration dose is low and it is non-toxic for humans. Although some freshwater fish might be affect [85].

Further methods are behavioural modification by education and training of the local population for example minimizing fresh water contact, wearing of footwear and other protective clothing or use of DEET (Diethyltoluamid) insect repellent after freshwater contact [102]. Other important factors are improvement of sanitation and the availability and use of clean water sources.

1.1.7 Treatment of *Schistosoma haematobium* infection sequelae

In more severe cases pathologies caused by *S. haematobium* infection are not reversible after PZQ administration and require specific treatment.

Hydronephrosis

When chronic obstruction of the ureter leads to hydronephrosis, which is not reversible through anti-schistosomal therapy, surgical intervention may be necessary to prevent further damage of the renal tissue. Polymeric double pigtail ureteral stents are a commonly used option. The main disadvantage of these stents is the need for periodic exchange. There is a variety of stents of different materials and coatings available, which will not be discussed in detail here. Further options are extra-anatomic bypass or other methods to ensure alternative urine drain [103].

Schistosomal bladder cancer

The standard management of schistosomal bladder cancer in a non-metastasized stage is a radical cystectomy with consecutive lymph node dissection. A study from Egypt showed that after 10 years, disease-free survival was 50.03%. Variables that affected survival probability were lymph node status, histological grade and tumour stage [29]. Adjuvant as well as neo-adjuvant radiotherapy is being discussed while data for the adjuvant radiotherapy is more promising [104, 105]. The use of chemotherapy has only been assessed in smaller trials but may also be beneficial [106]. Further and newer data is required. Metastasized stages should be referred to palliative care.

1.2 Point of care ultrasound

General aspects of ultrasound

As also described by Ihnatsenka and Boezaart [107] ultrasound has been used in medical diagnostic since 1942 and is applied in several medical fields. The technique is based on the effect that interfaces have on the reflection of sound waves. The ultrasound machine's probe directs longitudinal sound waves that travel through a medium (human tissue) by temporary compression and dilution of the medium's molecules. Different media like water or air have diverging echogenicity, which means that they reflect or transmit sound waves in different intensities. An interface between two media/tissues of a diverging echogenicity will appear as a change in contrast on the ultrasound machine's screen.

White structures are called hyperechoic, grey ones hypoechoic and black surfaces are anechoic structures.

There are high and low frequency probes. High frequency probes provide good resolution but less penetration while low frequency probes have a deeper penetration with poorer resolution.

In contrast to for example X-ray, ultrasound does not use ionizing radiation and therefore has no radiation exposure. It is also safe to use ultrasound in pregnant women and children, although here short exams are recommended since part of the sound waves is absorbed which means that the energy is converted into

heat. Additionally, ultrasound provides images in real-time and shows movement of organs or blood flow. Since comparably cheap and good quality ultrasound machines are available today, it is a cost-effective and non-invasive imaging technique, which can be used in almost every medical field. The major disadvantage of ultrasound is that quality of images is examiner-dependent and may therefore vary.

Point-of-care ultrasound

Point-of-care ultrasound (POCUS) is defined as an ultrasound exam performed and evaluated at the patient's bedside by the clinician in charge of the patient's management. It can be used for procedural guidance, as a screening or a diagnostic tool. The concept comprises a limited number of defined and easy to find ultrasound views as well as one or more defined (usually "yes/no"-) questions for each view.

Since ultrasound is examiner dependent, it is not recommended as a screening tool in the general population without specific symptoms. False positive results can lead to harmful and unnecessary interventions. Nevertheless, it can be a very useful tool for screening in specific subgroups. POCUS as a diagnostic tool is most established in emergency medicine. FAST, (focused assessment with sonography for trauma) is a widely used assessment to rule out internal bleeding in blunt trauma patients [108].

Point-of-care ultrasound in tropical medicine

Due to the availability of affordable good quality ultrasound machines, the POCUS approach has also been successfully introduced within various medical specialties in resource-limited regions including the diagnosis of tropical infectious diseases. The two most applied POCUS modules are focused assessment with sonography for HIV-associated tuberculosis (FASH) and focused assessment with sonography for echinococcosis (FASE). These modules allow also less experienced sonographers to detect signs that are highly suggestive for these infections in endemic regions. In a recent review, the

general use of POCUS in other diseases such as schistosomiasis, viral haemorrhagic fever or lymphatic filariasis have been suggested [109, 110].

Point-of-care ultrasound in urogenital schistosomiasis

While most countries in which *S. haematobium* is endemic have addressed the morbidity control by implementing governmental MDA programs, more severe pathologies, which cannot be cured by PZQ intake alone anymore, are still widely missed due to a lack of diagnosis.

While the Niamey protocol has fulfilled its primarily purpose to make scientific data more comparable and has found great acceptance in the scientific context, until today it has hardly been used in everyday medicine in endemic areas [77]. One of the main reasons for this is the lack of experienced sonographers in these regions who are able to apply the protocol.

1.3 Rational of the focused assessment with sonography for urinary schistosomiasis study

This problem was addressed in this study by simplifying the Niamey protocol to a POCUS module for urinary schistosomiasis morbidity detection called focused assessment with sonography for urinary schistosomiasis (FASUS). The goal here was to develop ultrasound as a cheap and non-invasive tool for everyday medicine in endemic countries and thus help to improve management of bladder and kidney pathologies due to *S. haematobium* infection.

The primary aim of this study was to evaluate the accuracy of the FASUS protocol, when applied by an examiner with no or little prior sonography experience compared to the gold standard of a remote review performed by an expert radiologist. If this technique proved to be an easy to learn and accurate tool to detect urinary tract pathologies by inexperienced users of ultrasound, it offers a useful method to integrate the diagnosis of *S. haematobium* infection sequelae into the daily clinical routine in endemic areas or even to set up extensive screening programs to identify affected patients and provide further care.

2 Methods

2.1 Study design and objectives

The FASUS study was a prospective diagnostic study that was conducted in the semi-rural area of Lambaréné, Gabon.

The objective was to evaluate the feasibility and accuracy of the newly developed FASUS protocol to diagnose urinary tract morbidity related to UGS. Lambaréné and its rural environment are known to have a high prevalence of *S. haematobium* infections [111]. At the time the study was conducted there was no local infection control program executed efficiently (Bertrand Lell, direct communication) [112]. Thus, a high prevalence of untreated UGS was expected among the local population, making the area a suitable environment to conduct the FASUS study.

Primary endpoint was the diagnostic accuracy of the FASUS method to determine presence or absence of UGS-related urinary tract morbidity in patients symptomatic of UGS against the gold standard of a remote review of the ultrasound scan by an expert sonographer/radiologist.

Secondary endpoints were:

1. Accuracy of FASUS in the detection of specific UGS-related urinary tract pathologies, such as bladder wall thickening, bladder masses and hydronephrosis compared to the gold standard of expert agreement.
2. Accuracy of FASUS in the monitoring of UGS-related urinary tract morbidity one and three months after PZQ treatment against the gold standard of a remote review.
3. Correlation of FASUS findings with history of PZQ treatment, clinical presentation, urine dipstick findings and quantitative egg excretion, and *schistosoma* Polymerase chain reaction (PCR) on urine.
4. Comparison of patient management decision taken based on clinical and laboratory assessment versus patient management recommended following FASUS assessment.

5. Immunological and metabolic profile associated with UGS.

Point five is not subject of this thesis and will not be discussed.

2.2 Study settings

2.2.1 Study period

The active study period of the FASUS study was from December 2015 to June 2016. The laboratory procedures where finished in August 2016. The statistical analyses were completed 2019.

2.2.2 Study site

Gabon is a sub-Saharan country located at the African west coast right at the equator. The capital is Libreville. According to the World Bank Group 2018 [113] the country's total area is 257.670 km^2 with almost 90% covered by rainforest. Gabon is a presidential republic and as a former French colony the official language is French. There are over 40 other local languages spoken [114]. The country has a gross domestic income (GDP) of 16.658 billion (2019). This makes Gabon an upper-middle income economy. The countries' main source of income are its' oil deposits. Despite this, 24.4% of people in Gabon are living on $3.10 a day and 8% even from $1.90 or less a day [113]. The Oxford Poverty and Human Development Initiative determined the Multidimensional Poverty Index for Gabon in 2012 with 16.5%, which puts it on one level with Iraq (13.3% in 2012) and Morocco (15.6% in 2011) [115].

In 2008, the government launched a "*National Insurance and Social Welfare Fund (Caisse Nationale d'Assurance Maladie et de Garantie Sociale, CNAMGS*)" because of the rising number of AIDS-deaths (Acquired immune deficiency syndrome) [114, 116]. This insurance covers 100% of maternal care and reimburses 80-90% of general medical care cost [117]. However, registration still poses a problem for many people and especially in rural regions accessibility and quality of health care is therefore often still inadequate.

According to WHO estimates from 2016, 183.767 individuals in Gabon were in need of annual schistosomiasis-treatment, 90% (165.289) of them being SAC. A countrywide program for mass treatment with PZQ has been started in 2016. Based on Gabon's annual report from 2016, 67.425 individuals received treatment that year, which presents a national coverage of 36.7%. Of the treated individuals 58.768 were SAC, which equals a national coverage of 35.6% for SAC [112, 118].

Centre de Recherches Médicales de Lambaréné

The main study site was the *Centre de Recherches Médicales de Lambaréné (CERMEL)* in Lambaréné, Gabon. The *CERMEL* is a medical research facility based in Lambaréné, a town in a semi-rural area in the Moyen-Ogooué province of Gabon. It is located next to the *Albert Schweitzer Hospital* (*HAS*) and was built in 1981 as part of the third generation of the *HAS* to research tropical diseases within Gabon [119]. In 1991 the German researcher Prof. Dr. Peter Kremsner became head of the research laboratory and set the main research focus on the tropical diseases malaria and schistosomiasis. Five years later Prof. Kremsner also became director of the Tropical Institute of the *University of Tübingen* in Germany and established the strong cooperation between the two institutes, which still is very important today.

Since 2001 the *CERMEL* is administratively and financially independent from the *HAS*.

The *CERMEL* has partnerships with universities in the Republic of Congo and Cameroon, Vienna, Amsterdam, Leiden and cooperates with *"Österreichischer Auslandsdienst*" and the "*Vietnamese-German Center for Medical Research"* [119].

Previous studies on various aspects of schistosomiasis have been conducted at the *CERMEL* during the last years [111, 120, 121]. Therefore, the laboratory procedures needed for the FASUS study had already been established on-site and the study team had access to unpublished epidemiologic data on the local distribution of UGS.

The *CERMEL* includes five laboratories: the clinical laboratory, parasitology laboratory, the research/immunology laboratory, the tuberculosis laboratory and a microbiology laboratory. The urine and blood samples of the FASUS study were mainly analysed in the parasitology (urine dipstick, standard urine filtration and microscopy and preparation of urine for PCR) and the research/immunology lab (PCR, preparation of blood and urine samples for shipment and later immunology/metabolomic analyses); in special cases additional tests were conducted in the microbiology or clinical lab (urine cultures and whole blood count).

Remote review sites

The other two FASUS study sites were remote review sites of two expert ultrasound reviewers. One paediatric infectious diseases specialist with four years of US experience, including research on FASH in South Africa, was based at the Department of Paediatric Pulmonology, Immunology and Intensive Care Medicine, Charité - Universitätsmedizin Berlin and the Berlin Institute of Health in Germany. The other reviewer, a clinical radiologist with over 20 years of ultrasound experience, including in East Africa, was based at the Liverpool School of Tropical Medicine and the Department of Radiology, Royal Liverpool University Hospital NHS Trust, United Kingdom.

2.3 Study population, in- and exclusion criteria

A samples size calculation, that was performed during the planning period of the study, showed, that in order to achieve 95% confidence and 80% power to detect a discordance of 10% of two diagnostic tests, a sample size of a minimum of 116 participants had to be included.

This calculation was made assuming a mean prevalence of UGS-related urinary morbidity of 30% in the study area (unpublished data, CERMEL).

The study population comprised adults and children of all ages living in Lambaréné and the surrounding area, presenting with symptoms compatible with UGS.

The inclusion criteria were a written informed consent and a history of symptoms compatible with UGS in the present or the past (reported macroscopic hematuria, dysuria, recurrent urinary tract infections (UTIs)).
Exclusion criterion was a known kidney and/or bladder disease related to other causes than UGS.

2.4 Development of the study protocol

The first step in the design of the FASUS study was the development of a simple and easy-to-learn ultrasound protocol to detect UGS-related pathologies in bladder and kidneys. The US protocol was developed by part of the study team and based on the guide for the detection of schistosomiasis-related morbidity with US by the WHO, often referred to as NIAMEY protocol [80].
This protocol was developed to provide a standard in the evaluation of renal pelvis, ureters and urinary bladder and comprises complex procedures such as the calculation of a severity grading score. While the Niamey protocol was developed for expert sonographers, the FASUS protocol aimed to be an easy-to-learn method for physicians in remote tropical settings without any prior ultrasound education, feasible with low quality ultrasound devices. It should however deliver enough information to the examiner to be able to judge morbidity of the respective patients. The original FASUS protocol comprised five scans: one transversal bladder scan, one longitudinal and one oblique scan of each kidney. Later a longitudinal scan of the bladder was added to the protocol because an added value in detection of pathologies in the bladder dome was suggested.

2.5 Study procedures

Figure 1 shows the study procedures in a chronological order. The procedures highlighted in dark grey were only performed for part of the study participants. The two main aspects: Radiologic procedure and Laboratory procedures are described below.

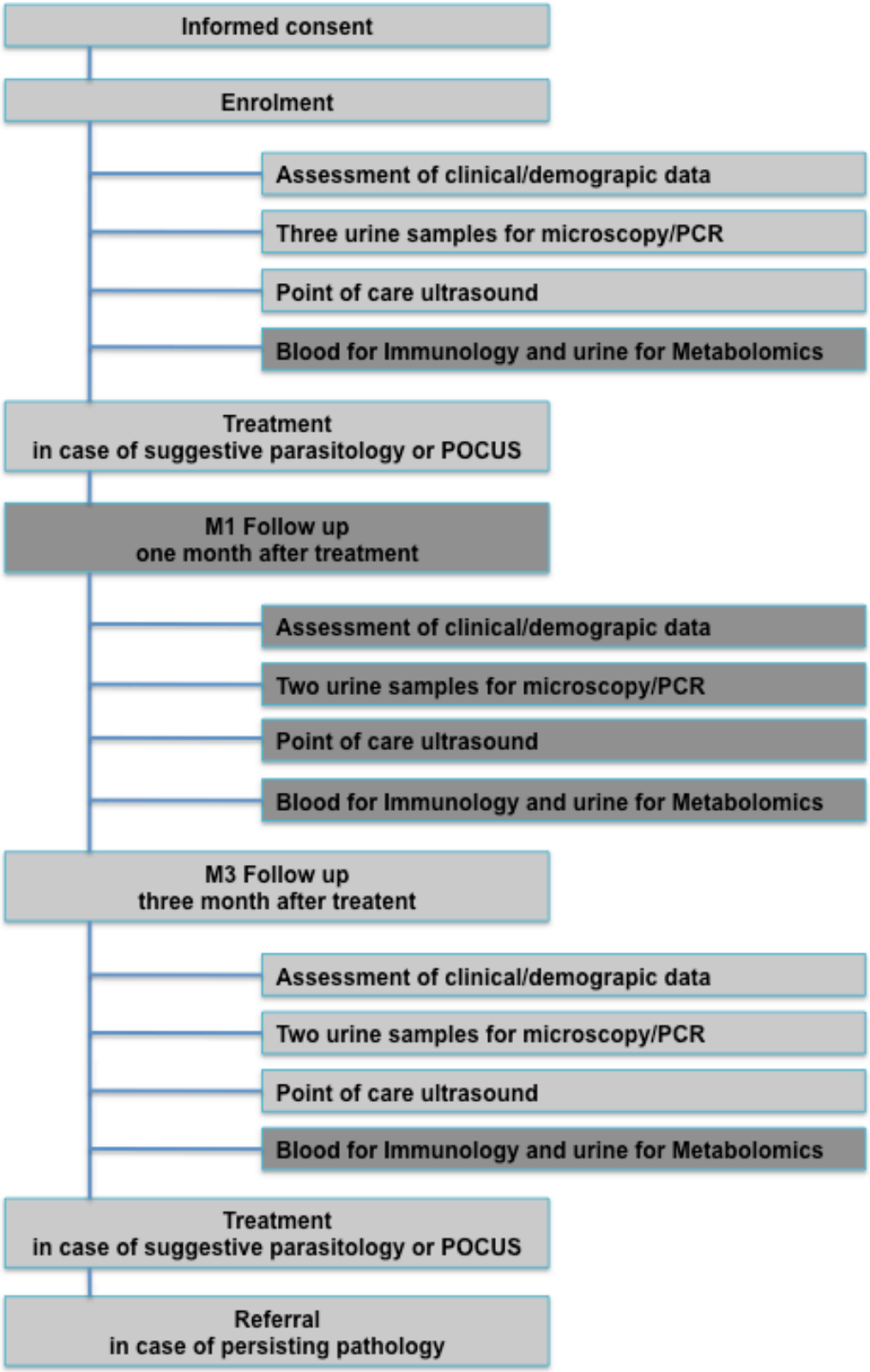

Figure 1: Flowchart of the FASUS study procedures, procedures highlighted in light grey were performed for all patients, procedures highlighted in dark grey were only performed for patients recruited after the amendment; POCUS: point of care ultrasound; PCR: polymerase chain reaction.

2.5.1 Training of the local investigators

Before inclusion of the first patient, the local investigators were trained in the FASUS ultrasound method. The first ultrasound investigator was a recently graduated clinician from Germany (operator 1) with limited experience in ultrasound examinations. A theoretical training comprised the study of ultrasound literature such as the WHO Ultrasound Manual [122], a variety of articles on schistosomiasis, as well as several exemplary images showing different schistosomiasis related pathologies in kidney and bladder in addition to the standard operating procedure (SOP) of the FASUS protocol. The theoretical training focused mainly on general point-of-care ultrasound aspects including safety questions, UGS-related morbidity and pathology, the context and aim of the FASUS protocol, the technical setup and requirements needed to perform FASUS, the FASUS standard scans to be obtained, the interpretation of sonographic findings obtained and the reporting and documentation of FASUS.

The ensuing practical training comprised of several test scans in healthy volunteers, remotely evaluated by the two reviewers. Detailed feedback on probe-positioning, gain modification, depth and focus adaptation, speed and accuracy of the organ sweeps was submitted via email. After five test-scans with good results the preparatory training was completed. During the first recruitment period a fast feedback was granted to ensure that potential deficits in depth, gain and focus could quickly be corrected. Later on feedback time extended due to organisational reasons.

The training of the second, ultrasound-naive investigator (a third year medical student from Germany, operator 2) was conducted during the course of the study in the same manner. Additionally, operator 1 supervised operator 2 during the first ten scans. Operator 1 performed all of the enrolment scans and operator 2 all follow up scans.

2.5.2 Recruitment

The majority of participants were recruited actively by convenience sampling at UGS-hotspots in Lambaréné. Additionally, several patients were recruited in the *HAS* as well as participants of other studies conducted at *CERMEL,* who

reported symptoms of UGS. The recruiting and enrolment period started on December 10th 2015 and ended on March 8th 2016.

Obtainment of written informed consent and assent

Before participants were included into the study the rationale of the study and all procedures were verbally explained in detail to every patient or legal guardian. Additionally, an information leaflet was handed out to every family. A written informed consent was obtained from all patients before enrolment. For minors under 18 years, the parent or legal guardian had to give their written consent. For illiterate patients the fingerprint was taken as well as the signature of a witness who assured that the informed consent form was read out loud to the patient and was understood by the person in question. Participants were free to withdraw from the study participation at any time point without giving any reasons.

2.5.3 Enrolment

After written informed consent had been obtained, a detailed questionnaire about the symptoms of UGS was applied. All patients were examined with US and urine samples for parasitological examination were collected.

Later in the course of the study an amendment was applied to the protocol and additional urine (for metabolomics analysis) and blood (for immunological analysis) samples were collected from a certain group of patients. Details are specified below.

Assessment of demographic and clinical data

The participants were interviewed by one of the investigators regarding presence and duration of macro-hematuria, dysuria and other UGS symptoms related to their sex (male: testicular swelling, hematospermia; female: urethral discharge, itching). Furthermore information about recurrent UTIs, any known bladder or kidney diseases, duration of freshwater contact and last PZQ intake were documented. Women were additionally assessed regarding their pregnancy status.

Collection of Urine samples

At enrolment urine was collected from all patients in order to determine possible *Schistosoma haematobium* infection as well as intensity of the infection via ultrafiltration and microscopy (details in section 2.5.13). To maximise diagnostic accuracy and minimise false negative results, collection of three urine samples on consecutive days was attempted [57].

Urine containers (50 ml) were distributed to the patients or parents/legal guardians the day before or directly at collection time. A minimum of 20 ml of urine was requested. If possible, urine samples were collected between 10 AM and 2 PM, as this was proven to be the time of the highest egg excretion rate [123, 124].

Ultrasound procedure

Every included patient was examined by sonography for UGS morbidity using the FASUS protocol as described in the methods section.

2.5.4 The Amendment

As a pilot study the FASUS protocol underwent a couple of changes during the course of the study to optimize the procedures in order to generate as much high quality data as possible under the given circumstances.

The first version of the FASUS protocol was revised in December 2015 after 26 patients had already been included. The amendment comprised changes in the enrolment procedures as well as the follow up.

According to the initial protocol participants had to provide three urine samples for parasitology and undergo the US examination at enrolment. Patients with urine samples positive for *S. haematobium* by microscopy were treated with PZQ.

All patients with US findings “compatible with UGS” by expert agreement were scheduled a follow up visit three months after treatment (M3). M3 follow up procedure was identical to the one at enrolment and patients with persisting positive parasitology or US scans still found “compatible with UGS” were treated a second time with PZQ.

The amended protocol was applied to all participants following recruitment number 27. Just as before three urine samples for parasitology were collected and the point-of-care ultrasound was performed at enrolment. Additionally one morning urine sample (before 10 AM) for metabolomic analyses was collected, as well as 10ml of heparin blood for immunological analyses.
All patients, recruited after the amendment (regardless their US results), were scheduled two follow up visits: one (M1) and three month (M3) after treatment, respectively. In case of persisting egg excretion or US pathology at follow up M3, patients were treated with PZQ again.

Collection of Blood and urine samples after the amendment
According to the amended protocol, 10 ml of heparin blood were drawn from all patients over five years of age at enrolment and follow up visits. The blood samples were used for immunological analyses. All venepunctures were performed at the *CERMEL* to enhance sterile and safe circumstances.
Furthermore, an additional urine sample was taken in the morning before 10 AM (as far as logistically possible), directly put on ice and then processed in the research lab to be stored and subjected to metabolomics profiling in order to identify biomarkers of early pathology.

2.5.5 Treatment

After all urine and one blood sample at enrolment had been collected and analysed/stored and the US examination was conducted, all patients with at least one urine sample positive for *S. haematobium* or an US exam with pathologies “compatible with UGS” by expert agreement, were treated with a single dose of 40mg praziquantel / kg body mass. Based on the instructions given in the package insert of PZQ [83], a table with different weight categories and the respective PZQ dose was designed (Table 1).

Table 1: Praziquantel dosing table

Body weight (kg)	12-19	20-25	26-33	34-41	42-48	49-56	57-63	64-70	71-78	79-86
Number of tablets (600mg)	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5

Usually the treatment succeeded approximately two weeks after the first urine sample was collected. Contraindications for PZQ were assessed before administration.

2.5.6 Follow up

According to the initial study protocol, all patients with an expert-review suggestive of UGS-related urinary tract pathology or any other or unclear pathology were followed up once, three month after the initial treatment with PZQ.

After the protocol amendment had come to effect, patients were followed up twice irrespective to their US results to obtain a maximum of information on infection status and morbidity for later correlation with immunologic/metabolomic findings. The follow up time points were one and three months after the initial treatment.

Follow up procedure:

The follow up visits M1 and M3 took place either at the *CERMEL* or at the patients' residence. The study team performed the same clinical and diagnostic procedures as for enrolment. An ultrasound scan was done and the collection of two urine samples for parasitology, one urine sample for metabolomics and one blood sample were attempted (for patients after the amendment). Similar to enrolment, venepuncture was only performed in *CERMEL* facilities. Participants were informed about their follow up approximately five days before the due date. In case participants could not be found at their homes or reached by phone after several attempts, they were classified as lost to follow up.

2.5.7 Retreatment

Patients with persisting egg excretion at M1 did not receive retreatment, as the drug was expected to have not yet reached its maximum therapeutic effect yet.
Patients persisting with egg excretion at M3 as well as those with persisting UGS-related pathology in US were treated a second time with a single dose of 40mg/kg body mass of PZQ.

2.5.8 Handling of patients with persisting urogenital schistosomiasis-compatible lesions at the three month follow up

All patients that were still found positive for UGS-compatible lesions at M3 were administered a second dose of PZQ. Participants or parents/legal guardians were informed about the results and in case of lesions such as intermediate/serious hydronephrosis or advanced bladder lesions, a detailed report form was handed out and participants were referred to a urological specialist in Libreville.

2.5.9 Management of patients with pathological non urogenital schistosomiasis-related ultrasound findings

Patients with pathological FASUS findings likely not related to UGS were also followed up twice. In case the expert-reviewers identified persisting pathology suggestive of non-UGS related origin requiring further diagnostic or therapeutic intervention, the patient or parent/guardian was informed by the study team and given a detailed report form and assigned to a specialist (for example gynaecologist). In some cases additional exams (for example whole blood count, Hepatitis/HIV-test) were performed with agreement of the patient or legal guardian before the assignment to the specialist.
All medical decisions taken by the attending doctor or study physician that concerned treatment or further diagnostics of the study participants were documented in the case report form. These included antihelminthic and other treatments, referral to a specialist, and the necessity of follow ups.

2.5.10 Focused assessment with sonography for urinary schistosomiasis

All FASUS ultrasound examinations were performed with a portable US device (MINDRAY Digital Ultrasonic Diagnostic Imaging System model DP-10, Mindray Medical International Limited, Shenzhen, China) with a curved array transducer (MINDRAY model 35C50EB). The portable ultrasound machine was already on site due to a previous study.

Depending on the recruitment situation and logistical factors the ultrasound examinations were done at the patients' homes or the *CERMEL*. If scans were performed in private houses the patients or parents were requested to provide a mattress in a darkened room in order to be able to assess the US scans properly. To achieve good quality bladder scans, patients were asked to drink a reasonable amount of water 30 minutes to one hour prior to the US exam to reach sufficient bladder filling, defined as complete distension of the bladder by eye balling. Before the start of the US examination the procedure was elaborately explained to all participants, especially smaller children to avoid fear and achieve good compliance during the scan.

The ultrasound machine was set up at the right site of the patient and the settings were chosen as following: urology, 4.5 MHz, gain: 35, depth: 13.

The FASUS scan included one transversal and one longitudinal sweep through the urinary bladder, as well as each a longitudinal and oblique sweep through both kidneys.

The first position was the urinary bladder in a transversal scan. The probe was placed horizontally above the pubic symphysis with probe orientation projecting the right side of the patient on the left side of the screen. Axial sweeps through the bladder were performed to assess the shape and wall of the bladder, as well as the distal ureters. Depth and gain settings were adapted appropriately for anterior and then posterior bladder wall/ureters to avoid artefacts and reduced visibility of the bladder wall.

The second probe position was the urinary bladder in a longitudinal scan. The probe was now turned clockwise until one end pointed towards the head of the patient. Longitudinal sweeps through the bladder were performed assessing the same structures and pathologies as in the transversal scan.

Normal findings in the bladder would be a fully distended bladder with a regular, rectangular shape. A bladder wall of regular thickness and not thicker than 5 mm. Normal distal ureters are not visible. Possible pathological findings are described in Table 2.

After having performed several sweeps through the bladder, the best representative sweeps were stored as a digital CINE clip on the machine under the labels “bladder_trans” and “bladder_long”, respectively. In case of any pathologic findings additional still images with relevant measurements were stored (JPEG format). The findings were documented in the case report form (CRF) as described below.

The third probe position was over the right kidney in order to perform a longitudinal kidney scan. The patient was asked to put his or her right arm beside his or her head or across the chest. The transducer was positioned obliquely in the right lowest intercostal space in the mid-axillary line. The kidney can be visualized inferior to the right lobe of the liver, using it as an “ultrasound window”. To picture the longest craniocaudal diameter the probe was turned clock-wise or counter-clockwise. If necessary, turning the patient to the left can be helpful while placing the probe.

Number four was the axial view of the right kidney. The probe was now turned counter-clockwise until a transverse view of the kidney was seen. The probe was then moved up- and downwards on a craniocaudal axis sweeping through the renal hilum to assess the renal pelvis for dilatation.

For view five and six the same was applied for the left kidney.

Normal finding for the kidneys would be a hypo-echoic (dark grey) kidney periphery (the renal cortex and pyramids) and a bright (echogenic) central area of the kidney, the renal sinus, which consists of the calyces, renal pelvis and the renal sinus fat. The renal pelvis should measure < 1 cm in diameter on the axial view. The ureters should not be visual in ultrasound unless distended. Possible pathological findings are listed in Table 2.

Again the best representative sweeps were stored under the label “Kid R long” (ideally showing the longest craniocaudal diameter and the renal hilums) and

“Kid R ax”. Additional images with measures were stored in case of any pathologic findings. Subsequently the findings were reported in the CRF.
The exact standardized US methodology was specified in a dedicated SOP. The probe positions are illustrated in Figure 2.

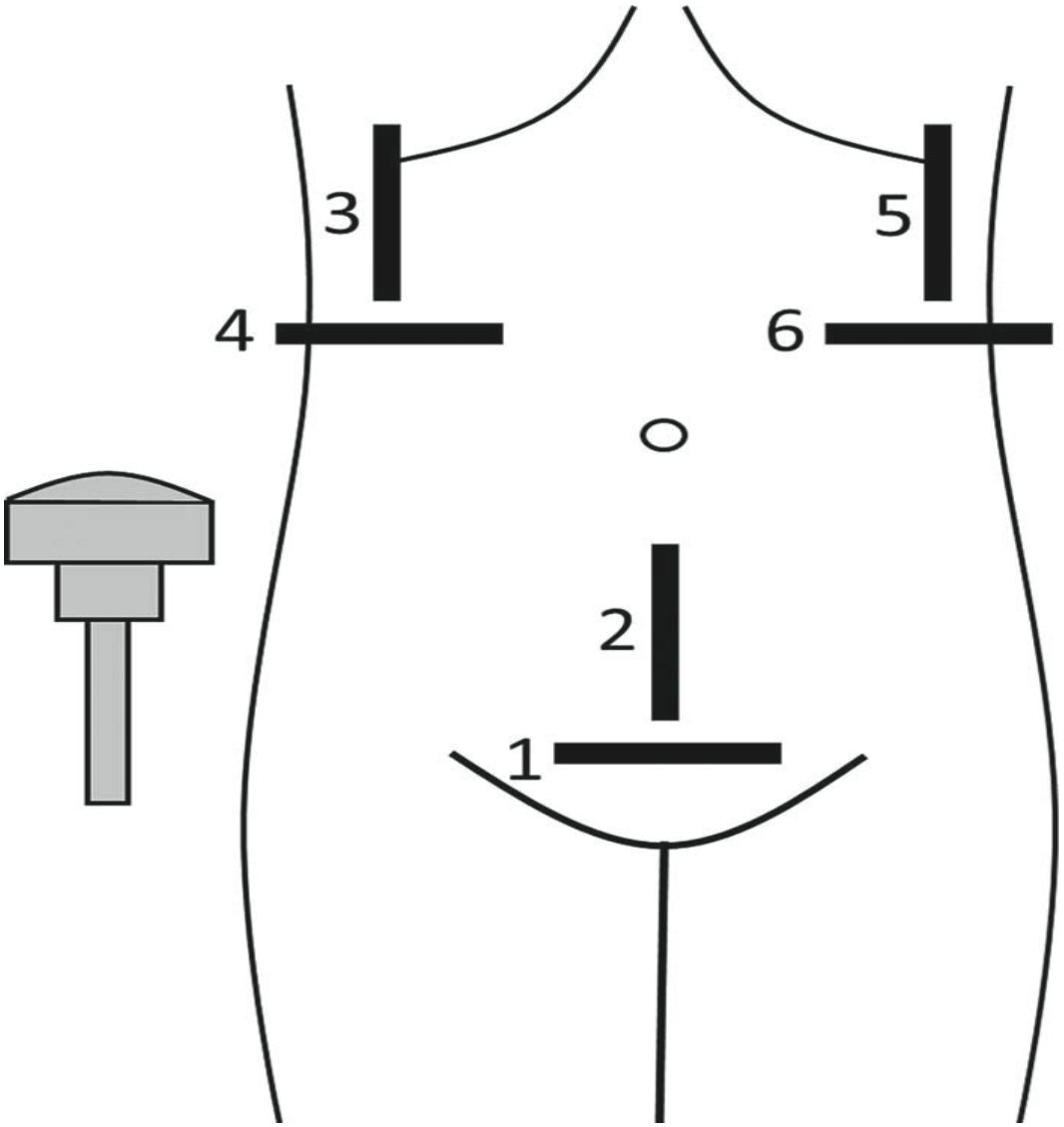

Figure 2: FASUS probe positions: 1 and 2 = transverse and longitudinal suprapubic pelvis scan; 3 and 4 = oblique and transverse right kidney scan; 5 and 6 = oblique and transverse left kidney scan. (Figure adapted from [125]).

Initially only one transversal bladder sweep was designated. During the course of the study the investigators assumed an additional value of a longitudinal

bladder-scan in the detection of bladder wall thickening especially at the bladder dome, though. Therefore an additional scan was included to the protocol and performed for all patients following recruitment number 45 at enrolment and for all follow up scans.

Documentation

The local operator recorded the US findings on a paper CRF. The main information assessed was bladder wall thickening and renal pelvis dilatation over one centimetre (for all options view Table 2). If renal pelvis dilatation was rated as moderate, it had to be confirmed after bladder voiding. Additionally the CRF enquired the examiners' opinion on the aspects of visualization with the options "satisfactory", "moderate" or "poor", normal bladder shape and ureter dilatation (proximal and distal) with the options "yes", "no", "unable to assess". Non-UGS related findings in bladder and kidneys were documented in the same manner. The last point summarized the examiners opinion about the exams result, posing the questions: "Pathology compatible with UGS" and "Pathology other that UGS". Optional answers were again "yes", "no" and "no interpretation possible.

Table 2 lists all six scans with the respective possible pathological findings. "Pathology compatible with UGS" was defined as bladder wall thickening ≥ 5 mm.

Table 2: FASUS ultrasound protocol (Table adapted from [125])

Scan planes	Assessed anatomy	Pathology variables[a]
Supra-pubic pelvis		
1. Transverse	• Bladder wall • Distal ureters	a. Bladder wall irregularities <5 mm thickness b. Wall thickening 5-10 mm c. Maximum thickening, masses or pseudo-polyps ≥ 10mm d. Bladder wall calcification e. Distal ureter dilatation or thickening f. Findings not related to urogenital schistosomiasis
2. Longitudinal[b]	• Bladder wall • Distal ureters	
Kidneys (right and left)		
3. and 5. Oblique	Proximal ureter and collecting system	g. Proximal ureter dilatation h. Findings not related to urogenital schistosomiasis
4. and 6. Transverse	Renal pelvis	i. Renal pelvis dilatation (>1cm) • If abnormal, confirmed after bladder voiding j. Findings not related to urogenital schistosomiasis

[a]any of b), c) or d) was defined as a pathology compatible with urogenital schistosomiasis.
[b]added to the protocol after recruitment of 45 cases because a higher sensitivity in the detection of bladder wall thickening was assumed.

The findings were documented directly after the scan in the CRF. If a second investigator was on site, the questionnaire was filled in simultaneously as instructed by the US operator.

2.5.11 Uploading procedure

The clips and images stored on the US device were transferred to an external hard drive via universal serial bus (USB) and then deleted from the ultrasound machine. Subsequently the Audio Video Interleave (AVI) format data was compressed using the open source video transcoder "*HandBrake*" (https://handbrake.fr; Version 0.10.2, released 6.9.2015) and then transformed into a zip-file using the program "*7zip*" (http://www.7-zip.org, version 9.38 (Beta) released 4.1.2015) in order to reach a smaller file size and be able to upload the files despite limited internet access and reduced data signalling rate. During the inclusion process the zip files were directly uploaded to the teleradiology platform "*collegiumtelemedicus*" (https://collegiumtelemedicus.org/ct/index.php) accompanied by some basic patient information (age, sex, history of symptoms, known illnesses and previous PZQ intake). Due to time limitations the uploading procedures was changed later on for the follow up scans. The local investigator in Lambaréné uploaded all files into a dropbox folder from where it was afterwards transferred to the teleplatform by a second investigator in Germany.

2.5.12 Remote review

The video clips and pictures on the teleradiology website were evaluated by the two expert reviewers. They documented their findings on a digital CRF in Portable Document Format (PDF) format, which was then uploaded to the telemedicine platform. This report form was adapted several times during the study to cover all necessary information and provide a fast and easy handling for the reviewers.

For all inclusion scans the experts had to rate the settings "gain", "focus" and "depth" with the options "good", "moderate" and "poor". For the two bladder views it was additionally asked for "bladder entirely swept", "bladder sufficiently

filled" and "normal bladder shape". For the kidney scans this was replaced by "anatomical orientation". Finally it was asked for "diagnostic quality of images" with the options "good", "moderate" and "poor" in all four sections.
For the follow up report forms detailed feedback was no longer needed and the detailed questions were replaced by just rating the "diagnostic quality of images".
In average, the experts completed the reviews two to four weeks after the scan was uploaded on the platform.

Expert discussions

In case the two reviewers had diverging opinions about "pathology compatible with UGS" or "pathology other than UGS" a discussion was scheduled. The discussion was held via Skype and the result was documented in a separate discussion report form.
Later on a second expert discussion was held in case of diverging opinions concerning distal ureter dilatation, proximal ureter dilatation and kidney pelvis dilatation.

2.5.13 Laboratory procedures

Every urine sample for parasitological analyses underwent the following diagnostic procedures:

Semi-quantitative urine dipstick

For dipstick testing the Combur® Test 10 was used (Roche Diagnostics Ltd, Risch, Switzerland). The test stick was dipped briefly but completely into the room temperature urine. After 30 seconds the results could be read. Only leukocyte, nitrite, protein and erythrocyte values were registered.

Parasitological testing:

Urine filtration and microscopy for the detection of *Schistosomia haematobium* eggs was performed according to the local SOP.

Ten millilitre of urine were filtrated through a milipore filter (Nuclepore Track-Etch Membrane Filtration Products, Whatman plc, Maidstone, UK) and then analysed under the "*Eclipse E200MV R*" microscope (Nikon, Tokio, Japan) using the 10x objective for presence of eggs of *S. haematobium* or *S. intercalatum*. An egg count over 50 eggs/10ml was rated as "heavy infection". If the egg count was 500 eggs/10ml or less the exact egg count was documented. For samples with more than 500 eggs/10ml urine the egg count was noted as ">500".

Urine conservation for polymerase chain reaction:

The remaining urine was conserved for possible analysis with PCR later on, following a methods applied in previous studies at the *CERMEL* [123].

Therefore the specimen was mixed thoroughly and 10ml were put into a 15ml CELLSTAR centrifuge tube and centrifuged for 5 minutes at 710 x g. Then 9.5 ml of supernatant was discarded and the rest (500µl) stored at -20°C in a 2ml micro-tube for later examination by real-time PCR.

DNA-extraction and polymerase chain reaction

To increase the diagnostic accuracy in the detection of *S. haematobium* egg excretion all samples found negative for *Schistosoma* eggs by microscopy during enrolment or follow up, underwent DNA extraction and PCR analysis.

As a preparation of the PCR analysis DNA extraction was performed. The "*protocol: DNA purification from blood or body fluids (spin Protocol) from QIAamp DNA Mini and Blood Mini Handbook 02/2015*" was used in a slightly modified version.

As a first step the two or three frozen urine samples that were available for the time point in question were mixed together, vortexed for 15 seconds and then centrifuged at 8000 rounds per minute (rpm) for one minute.

Then the supernatant was removed until a maximum of 0,75 ml left. If only one sample was available for the respective time point, the sample was also vortexed and then centrifuged.

Then 200 microliters of every sample were pipetted into a microcentrifuge-tube and then incubated in a water bath of 110°C for 10 minutes.

In the meantime, a mix of the AL Buffer and the Phocine herpesvirus (phHV) was prepared.
This was calculated as follows:
(200 x (number of samples+1)) µl of the AL Buffer was mixed with (0,5 x (number of samples+1)) µl phHV.
After the end of the incubation 20 µl of Proteinase k were added to each sample and vortexed. This mixture was incubated for two hours in a water bath of 55°C.
As a next step 200 µl of ethanol were added and the samples were vortexed again. The content of every microcentrifugation-tube was pipetted on a QIAamp Mini spin column within a collection tube and centrifuged at 8000 rpm for one minute. The QIAamp Mini spin column was placed in a new collection tube and the tube containing the filtrate was discarded. Then 500 µl AW1 buffer were added onto every column and again centrifuged at 8000 rpm for one minute. Subsequently the column was again placed in a new collection tube and the tube with the supernatant was discarded.
Then 500 µl of AW2 Buffer were added and centrifuged at full speed (12000 rpm) for three minutes. The tube with the supernatant was discarded and the samples were again centrifuged at full speed for one minute.
The columns were now placed in beforehand-labelled 1,5 ml microcentrifuge-tube and added 200 µl of AE Buffer. The tubes were incubated for one minute at room temperature and then centrifuged at 8000 rpm for one minute.
The columns were discarded and the tubes with the extracted DNA were put back into the -20°C freezer.

Immunological blood analyses and urine analysis for metabolomics

Since immunological blood analysis and urine processing for metabolomic analyses are not subject of this thesis, the respective procedures are not described in further detail here.

2.6 Data management and statistical analyses

Most data was recorded on paper report forms and kept in individual patient files on site. The digital report forms of the remote FASUS review were printed

out and kept in the patients' files. Data was entered into OpenClinica (OpenClinica® version 3.0.4, Boston, USA) by a local investigator and stored on the *CERMEL* server. Data analysis was done using Microsoft Excel (Microsoft, Redmond, WA, USA).

2.7 Ethical approval

The institutional ethics committee and the scientific review committee of *CERMEL* approved the FASUS study (reference number: CEI-CERMEL: 015/2015).

2.8 Safety and environment

All members of the study team working with urine or blood specimen followed general bio safety precautions applicable to a biosafety level 1 laboratory.

All spills of specimens or reagents were cleaned and disinfected using 70% Ethanol. All specimens, reagents and other potentially contaminated materials were decontaminated and disposed in accordance with local safety regulations of the *CERMEL*.

2.8.1 Confidentiality

All patient identities were pseudonymized by study numbers (FASUS XX), which were then used to label all laboratory samples and digital files.

2.8.2 Improvement of standards of care

The FASUS team also aimed to improve patient care regarding UGS in the study area and especially for all participants of the FASUS study. Therefore all results were reported to patients/legal guardians and all PZQ treatment was handed out for free.

Patients with findings not related to UGS received respective treatment from the study team or were referred for further management.

After the end of the follow up period the local investigators designed an information leaflet on Schistosomiasis infection and its prevention. This leaflet was based on the authorized use of the cartoon "*Bambo a la Bilharziose: ce que les enfants doivent savoir sur la bilharziose*" by the WHO and produced in cooperation and agreement with the ministry of health in Gabon [126]. The leaflet was handed out to all study participants and distributed in the recruitment area.

2.9 Good clinical practice

The study followed International Conference of Harmonisation of Good Clinical Practice (ICH-GCP) Guidelines and the Declaration of Helsinki.

2.10 Funding

There was no study specific funding. Resources other than consumables were covered within projects led by Michael Ramharter. Costs for ethics and treatment as well as PCR and immunology studies were covered by Ayola Akim Adegnika. Costs of consumables like urine containers, slides for microscopy, local investigators phone costs and ultrasound gel, were covered by *CERMEL*.

3 Results

A total of 119 participants were enrolled. One participant withdrew consent before the first FASUS scan was performed. Since the protocol was amended after enrolment of 26 participants (additional longitudinal bladder scan), all individuals enrolled thereafter were followed according to protocol 2.0 and were aimed to be followed up at M1 and M3. Follow up was done for 70 (59%) participants at M1 and for 82 (69%) at M3. Figure 3 pictures the flow of the FASUS study. The last follow up was performed on 16th of June 2016.

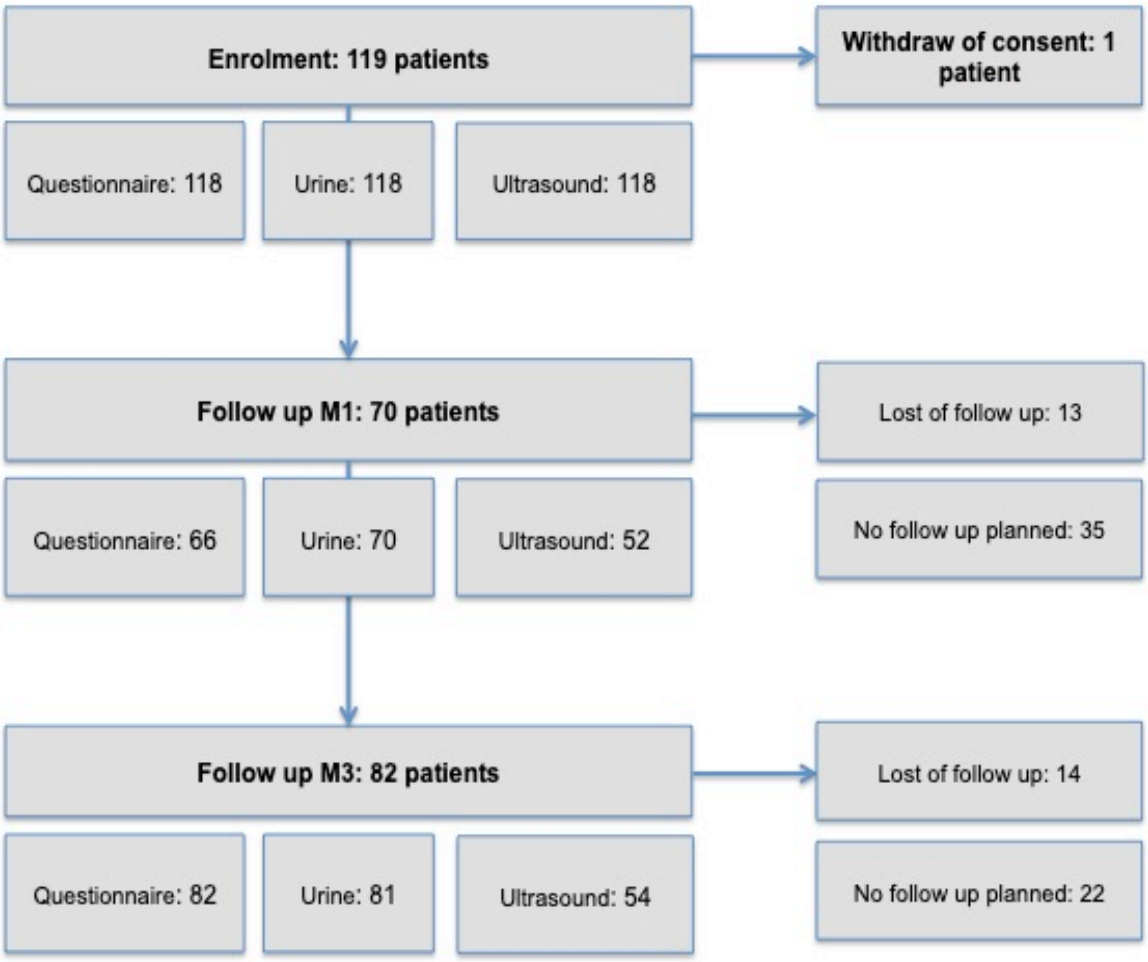

Figure 3: Flow and number of participants at enrolment and follow up; M1: follow up one month after treatment, M3: Follow up three month after treatment.

3.1 Demography

The median age of the FASUS study population was 11.2 years (Inter quartile range (IQR) 6.5-17.4), with almost half (48%) being SAC between six and fifteen years of age. Most of the study population was recruited in an area at the outskirts of Lambaréné called Petit Paris 3. The median duration of participants' fresh water contact before enrolment was three years.

The demographic data at enrolment is presented in Table 3.

Table 3: Demographic data of the FASUS study population at enrolment

Total number of patients		118
Sex	Female, n (%)	56/118 (48)
Age [a]	PSAC, n (%)	27/118 (23)
	SAC, n (%)	56/118 (48)
	Adults, n (%)	35/118 (30)
Residence	Moussamoukougou, n (%)	13/118 (11)
	Route Fangui, n (%)	31/118 (26)
	Petit Paris 3, n (%)	71/118 (60)
	Other, n (%)	3/118 (3)
Body mass adults [b,c]	Underweight, n (%)	1/18 (6)
	Normal weight, n (%)	14/18 (78)
	Overweight, n (%)	1/18 (6)
	Obese, n (%)	2/18 (11)
	NA, n (%)	2/20 (10)
Body mass children [d]	Underweight, n (%)	19/78 (24)
	Underweight – normal weight, n (%)	3/78 (4)
	Normal weight, n (%)	50/78 (64)
	Overweight, n (%)	1/78 (1)
	Obese, n (%)	5/78 (6)
	NA, n (%)	20/98 (20)
Previous PZQ* treatment	Yes, n (%)	50/118 (43)

[a] PSAC: pre-school children < 6years; SAC: school aged children 6-15 years; adults > 15years
[b] adults here defined older than 19 years
[c] Underweight defined as BMI (body mass index) <17,5; normal weight BMI 17,5 to 25; overweight BMI 25 to 30; obese BMI over 30
[d] children here defined as 19 years or younger using the WHO BMI-for-age (5-19 years) (http://www.who.int/growthref/who2007_bmi_for_age/en/) and (2-5 years) (http://www.who.int/childgrowth/standards/bmi_for_age/en/))
* PZQ: Praziquantel

In the group of SAC 32% children had received PZQ treatment within the last 12 month, most of them in the context of a national prevention program.

3.2 Clinical symptoms of urogenital schistosomiasis

Data on clinical UGS symptoms was collected using a questionnaire at enrolment and follow up visits. Clinical data at enrolment are presented in Table 4.

Table 4: Data on clinical symptoms of the FASUS study population at enrolment

		Enrolment
Total		118
Reported hematuria	Present, n (%)	93/118 (79)
	Previous, n (%)	25/118 (21)
	Median duration (IQR)	120 days (30-390)
Dysuria	Present, n (%)	56/118 (47)
	Previous, n (%)	12/118 (10)
	Never, n (%)	50/118 (42)
Recurrent UTIs[c]	Yes, n (%)	2/118 (2)
Hematospermia	Yes, n [a] (%)	1/11 (9)
Testicular swelling	Yes, n [a] (%)	6/53 (11)
Urinary incontinence	Yes, n [b] (%)	13/50 (26)
Vaginal discharge	Yes, n [b] (%)	10/45 (22)
Genital itching	Yes, n [b] (%)	28/54 (52)
	Median duration (IQR)	184 days (14-730)

[a] total number of males with available data concerning this symptom
[b] total number of female with available data concerning this symptom
[c] UTIs: Urinary tract infections

Considering the distribution of symptoms within the three age groups, it can be observed that both PSAC and SAC reported present hematuria in 85% of cases while in adults this was only 66%.

Six (60%) of the pre-school aged girls and 13 (65%) adult women reported symptoms suggestive for FGS (urinary incontinence, vaginal discharge and/or genital itching). In school aged girls FGS symptoms were only reported in 10 (38%) cases. Symptoms suggestive for MGS (hematospermia and/or testicular swelling) were reported by one (6%) pre-school aged boy and by two (13%) adult men. Among the school aged boys there were four (13%) cases with MGS suggestive symptoms.

3.3 Laboratory data

Data on the number of urine samples and results of urinary dipstick analyses at enrolment is presented in Table 5.

Table 5: Number of collected urine samples and dipstick results at enrolment

		Enrolment
Urine samples per patient, n (%)	One sample	4/118 (3)
	Two samples	21/118 (18)
	Three samples	93/118 (79)
Macrohematuria, n (%)	Yes	15/118 (13)
Dipstick[a]		
Patients with hematuria, n (%)	Yes	103/118 (87)
Intensity of hematuria, n (%)	1+	5/103 (5)
	2+	3/103 (3)
	3+	95/103 (92)
Patients with proteinuria, n (%)	Yes	87/118 (74)
Intensity of proteinuria, n (%)	1+	35/87 (40)
	2+	26/87 (30)
	3+	26/87 (30)
Patients with positive nitrit in urine, n (%)	Yes	20/118 (17)
Patients with leucocyturia, n (%)	Yes	87/118 (73)
Intensity of leucocyturia, n (%)	1+	20/87 (23)
	2+	32/87 (37)
	3+	35/87 (40)

[a] All figures present the highest value recorded in one or several samples of a patient for the respective parameter

The results of the microscopic examination for *S. haematobium* eggs in urine samples at enrolment are presented in Table 6.

Figure 4 shows infection and heavy infection rates in subgroups by age and sex.

Table 6: Results of the microscopic examination for *S. haematobium* eggs in urine samples at enrolment

		Enrolment
***S. haematobium* eggs in urine samples**	Positive patients, n (%)	105/118 (89)
Maximum egg count	<50 eggs, n (%)	36/118 (31)
	50-500 eggs, n (%)	32/118 (27)
	>500 eggs, n (%)	37/118 (31)
	Patients with no eggs, n (%)	13/118 (11)
Negative urine parasitology with positive PCR[a]		0
	Heavy intensity infection, n [b] (%)	69/118 (58)
By sex		
Females	Positive females, n (%)	50/56 (89)
Males	Positive males, n (%)	55/62 (89)
By age		
PSAC[c]	Positive pre-school children, n (%)	24/27 (89)
SAC[d]	Positive school children, n (%)	53/56 (95)
Adults	Positive adults, n (%)	28/35 (80)

[a] Polymerase chain reaction
[b] over 50 *S. haematobium* eggs / 10 ml
[c] Pre-school aged children
[d] School aged children

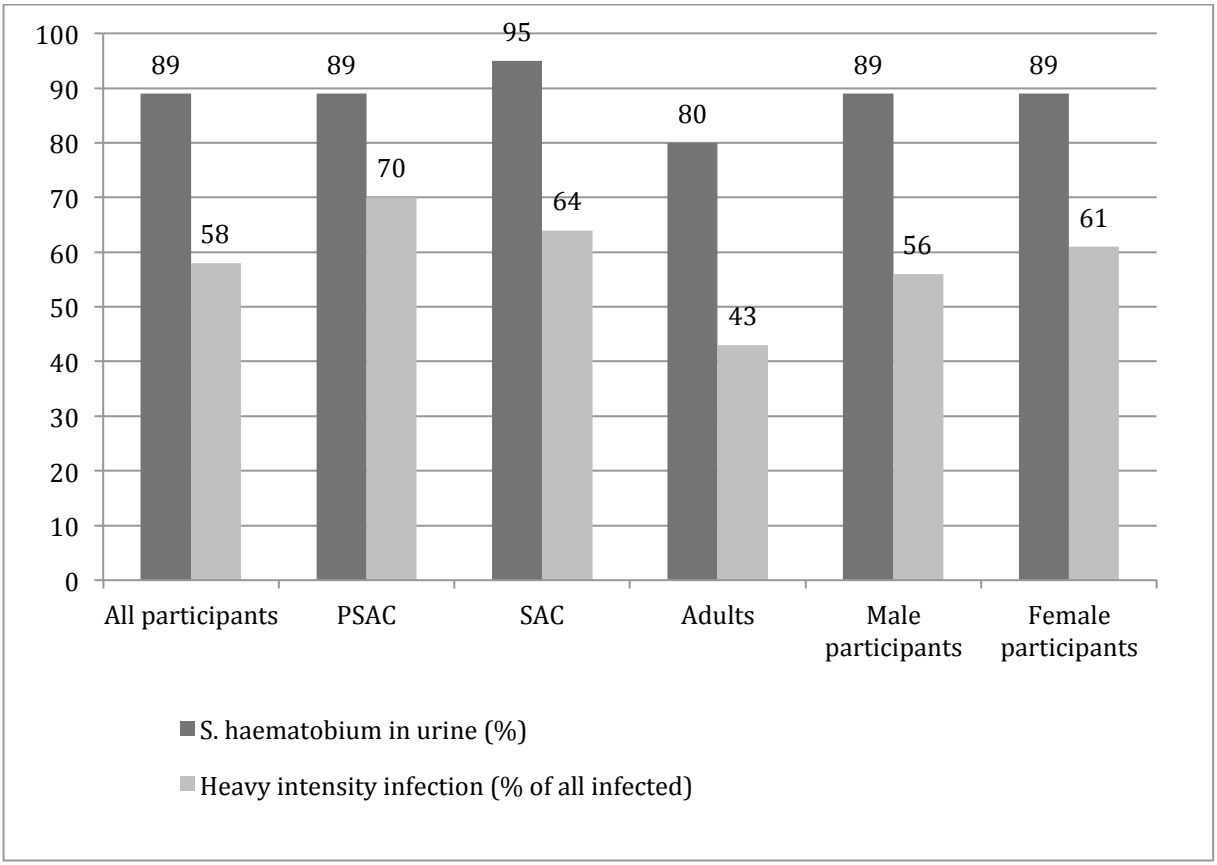

Figure 4: The proportion of heavily infected individuals in different sex and age groups compared to all individuals with S. haematobium infection in the respective subgroup of the FASUS study population at baseline; heavy intensity infection in percent of all infected individuals of this subgroup; PSAC: pre-school aged children; SAC: school aged children.

The examination of three urine samples per patient resulted in the detection of five additional cases of schistosomiasis at enrolment compared to the examination of only one sample per patient, while the second urine sample resulted in no additional detection. However, as the intensity of infection decreased after treatment, a larger gain could be obtained from the second urine sample during follow up. At M1, eight additional positive patients were discovered and at M3 seven additional patients.

There were no additional positive samples found by the PCR examination of the samples found negative in microscopy.

3.4 Image quality

Operator 1 performed all 118 enrolment scans and operator 2 all 106 follow up scans.

In average the FASUS ultrasound procedure took an estimated ten minutes according to the patients' bladder filling, compliance and body mass. The examination time varied between 10 and 35 minutes for the first examinations. Later on the time for a FASUS scan averaged five to 10 minutes.

Of the 224 scans that were performed during the FASUS study, 46 (21%) were done at *CERMEL* and 178 (79%) at patients' residences [125].

Table 7 presents an overview over the proportion of scans that were rated of sufficient diagnostic quality by expert consensus [125].

Of the scans performed at the hospital 3/46 (7%) were unable to be evaluated compared to 19/178 (11%) of home visit scans that were unable to be evaluated.

Table 7: Ultrasound image quality sufficient for expert interpretation (table adapted from [125])

		Bladder scan [a]		**Right kidney scan**		**Left kidney scan**	
Ultrasound operator [b]	**Total scans**	**Bladder wall**	**Distal ureters**	**Renal pelvis**	**Prox. ureter**	**Renal pelvis**	**Prox. ureter**
Operator 1, n (%)	118[c]	107 (91)	100 (88)	117 (99)	115 (97)	115 (97)	114 (97)
Operator 2, n (%)	106	95 (90)	93 (88)	106 (100)	103 (97)	104 (98)	104 (98)

Prox: Proximal.
[a] Combination of transverse and longitudinal scan, when available.
[b] Baseline scans performed by operator one, follow up scans performed by operator two.
[c] For distal ureter views at baseline: n = 114.

At baseline, image quality was sufficient for the diagnostic assessment of bladder wall pathology in 22 (81%) of PSAC, 54 (96%) SAC and 31 (89%) of adults, 59 (95%) of males, and 48 (86%) of females [125].

Image quality was rated “good” in more than 95% of transverse bladder and kidney scans. Image quality of longitudinal bladder scans was good in 78% and moderate in 20%. Depth was rated as good in 99%, gain in 98%, and focus in 98% of all scans. In 95% of sweeps the bladder was entirely displayed and sufficiently filled in 86%. In most cases of insufficient quality (7/11) the bladder was not filled properly. The kidney pelvis was displayed in 99% of scans and the anatomical orientation was rated good in 92% of sweeps [125].

With increasing number of performed US scans, determinants of image quality improved for both US operators. Operator 1 bladder scans improved from 85% sufficient quality for the first 59 US scans up to 97% in the following 59 scans at enrolment. Operator 2 also improved in the image quality of bladder scans from 89% for the first 53 scans to 91% in second half. Sufficiency of kidney views also improved slightly for both operators. Operators’ improvement in bladder wall assessment is shown in Figure 6 below.

3.5 Diagnostic accuracy of ultrasound findings

Diagnostic accuracy of pathology detection of the two operators performing FASUS against the reference standard of a remote expert review is presented in Table 8.

When adding up baseline and follow up scans operators missed 21/202 (10%) pathologies that were rated as compatible with UGS by the expert agreement. In 9/202 (4%) cases the patients were over-diagnosed by the operators.

For both operators renal pelvis dilatation had the highest agreement rate with the experts. It was lowest for distal ureter dilatation, with experts detecting it three times more often than the operators [125].

Table 8: Diagnostic accuracy of FASUS at enrolment (1) and follow up (2) by comparing operators' ultrasound findings with expert consensus[a] (Table adapted from [125])

	Bladder wall thickening ≥ 5mm		**Distal ureter dilatation/ thickening**		**Proximal ureter dilatation**		**Kidney pelvis dilatation >1cm[c]**	
Operator	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Total scans[b]	107	95	87	93	111	102	110	104
True positives	38	27	2	3	4	1	6	5
True negatives	55	52	73	79	104	95	102	98
False positives	6	3	2	1	0	5	2	0
False negatives	8	13	10	10	3	1	0	1
Sensitivity (%)	83	68	17	23	57	50	100	83
Specificity (%)	90	95	97	99	100	95	98	100
Inter-rater agreement (Cohen's Kappa)	0.73 "good"	0.64 "good"	0.19 "poor"	0.31 "fair"	0.71 "good"	0.23 "fair"	0.85 "very good"	0.90 "very good"

[a] Scans at enrolment performed by operator 1, scans at M1 and M3 follow up performed by operator 2

[b] all scans of sufficient image quality for interpretation by operator and expert consensus

[c] if confirmed after bladder voiding or severe

Ultrasound pathologies detected by experts and operators at enrolment are presented in Figure 5.

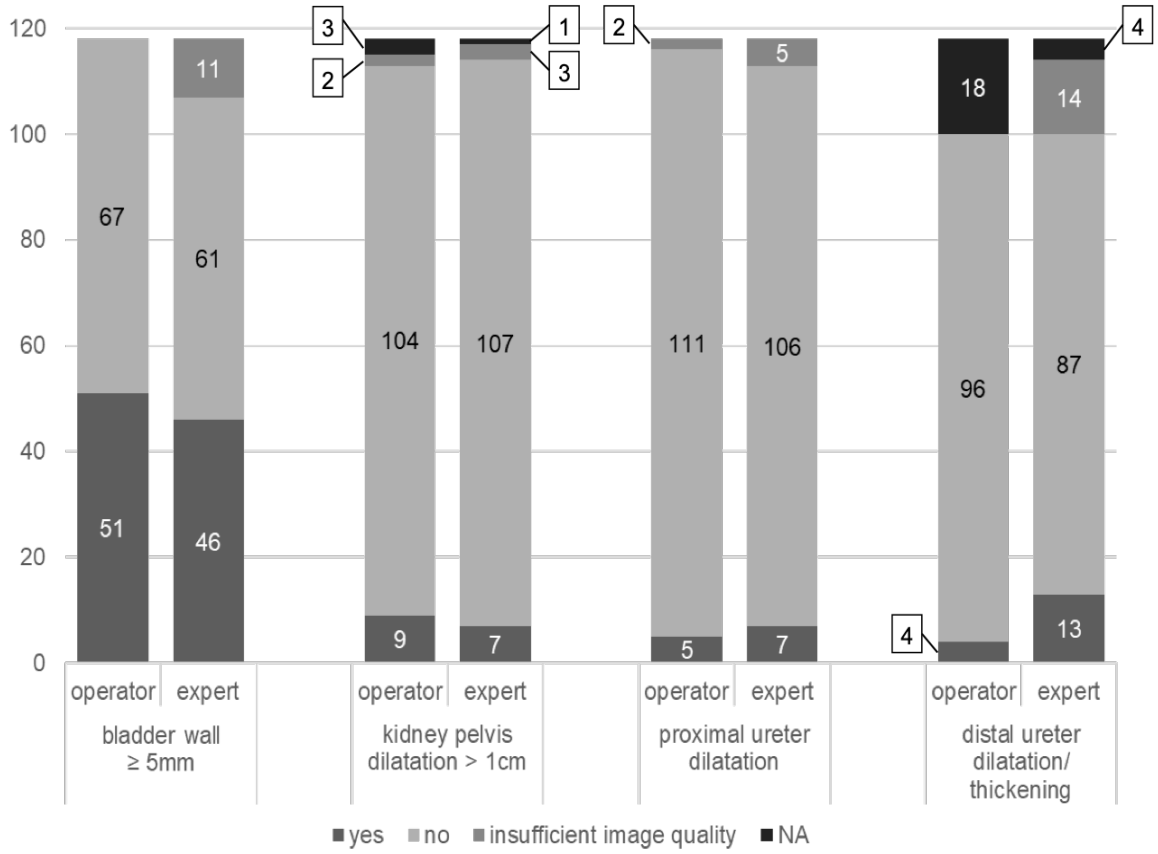

Figure 5: Pathology detected by FASUS at enrolment by operator and expert (figure adapted from [125]); NA: not available.

Figure 6 summarizes the development of image quality, sensitivity, specificity and Cohen's Kappa (k) in bladder wall pathology ≥ 5 mm for both operators compared to expert agreement over the increasing number of performed scans.

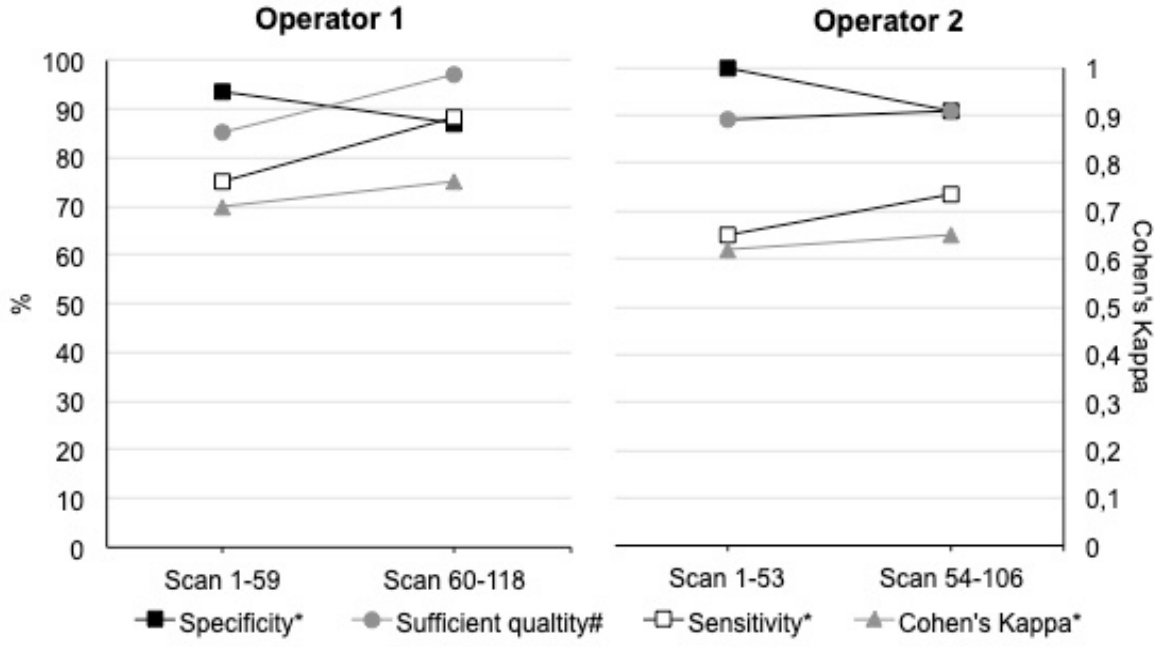

Figure 6: Operator improvement in bladder wall assessment (figure adapted from [125]); #image quality of bladder scans sufficient for expert interpretation in %; * ultrasound operator findings compared with expert consensus after remote review of recorded clips in %.

3.6 Expert ultrasound findings

Of the 118 ultrasound examinations performed at enrolment 107 (90%) scans were rated as interpretable by expert agreement. According to expert agreement 43% of them showed pathology compatible with UGS (any bladder thickening ≥ 5mm).

One patient with bladder wall thickening ≥ 5mm was negative for *S. haematobium* eggs in urine. In five patients renal pelvis or ureter dilatation was found in absence of bladder wall thickening of ≥ 5 mm, but all had microscopy positive urine parasitology. Of these patients three had bladder wall irregularities and one was pregnant [125].

The distribution of bladder wall thickening per demographic and laboratory data is shown in Table 9.

Table 9: Expert agreement on bladder wall thickening ≥ 5mm at enrolment for the different subgroups of the FASUS study

		Bladder wall thickening ≥ 5mm	**Interpretable scans**
	All patients, n (%)	46/107 (43)	107/118 (91)
Sex	Females, n (%)	21/48 (44)	48/56 (86)
	Males, n (%)	25/59 (42)	59/62 (95)
Age	PSAC[a], n (%)	9/22 (41)	22/27 (81)
	SAC[b], n (%)	29/54 (54)	54/56 (96)
	Adults, n (%)	8/31 (26)	31/35 (89)
Hematuria in dipstick	1+, n (%)	0/5 (0)	5/5 (100)
	2+, n (%)	1/3 (33)	3/3 (100)
	3+, n (%)	43/85 (51)	85/95 (89)
	No hematuria, n (%)	2/14 (14)	14/15 (93)
Maximal egg count	< 50, n (%)	6/35 (17)	35/36 (97)
	50-500, n (%)	13/29 (45)	29/32 (91)
	500+, n (%)	26/32 (81)	32/37 (86)
	No eggs, n (%)	1/11 (9)	11/13 (85)
Previous PZQ[c] intake	Yes, n (%)	16/44 (36)	44/50 (88)
	Never, n (%)	30/63 (48)	63/68 (93)

[a] PSAC: Pre-school aged children
[b] SAC: School aged children
[c] PZQ: Praziquantel

The longitudinal bladder scan was added after enrolment of the 26th patient and thus performed in 85 of 118 patients at enrolment. At follow up both scans were performed for all participants.

Among those 85 cases that were assessed by both scans, pathology compatible with UGS was found in 35/85 (41%) cases by transverse scan alone

and 36/85 (42%) by longitudinal scan alone. By combining both scans, pathology compatible with UGS was detected in 38/85 (45%) cases [125].
Types of bladder wall pathology detected by expert agreement at enrolment are presented in Figure 7.

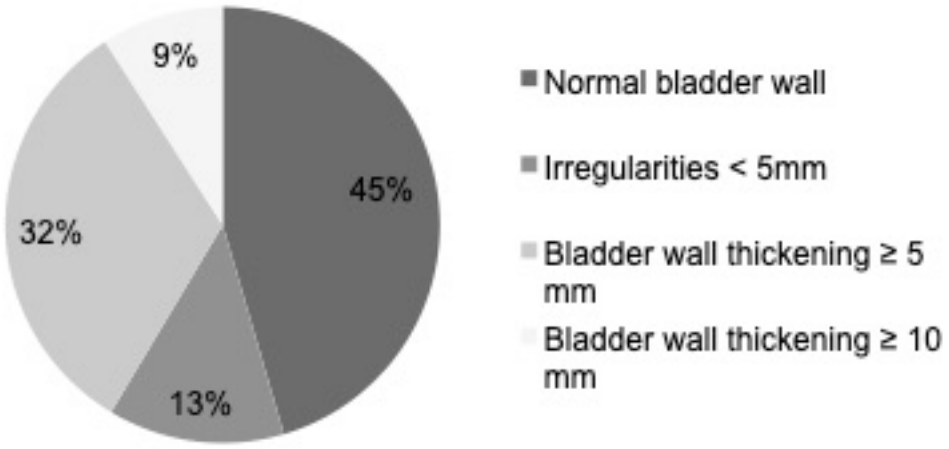

Figure 7: Types of bladder wall pathology by expert agreement at enrolment in the scans of sufficient quality (n=108).

At enrolment 19% (19) of 98 interpretable scans showed any upper urinary tract (UT) morbidity, which was defined as kidney pelvis dilatation >1cm (if confirmed after bladder voiding or severe) and/or proximal or distal ureter dilatation. For distal ureter dilatation only 114 scans were assed with 98 found interpretable by expert agreement. Four participants were not assessed regarding upper urinary tract morbidity and therefore are not included in this category.
Upper urinary tract pathology was found most frequently in SAC and 89% of patients with upper urinary tract pathologies suffered from heavy intensity infection with *S. haematobium*.
The enrolment data on expert upper urinary tract findings is presented in Table 10.

Table 10: Expert agreement on upper urinary tract findings at enrolment

		Any upper UT pathology[a]	Interpretable scans
	All patients, n[b] (%)	19/98 (19)	98/114 (86)
Sex	**Females, n[b] (%)**	9/46 (20)	46/55 (84)
	Males, n[b] (%)	10/52 (19)	52/59 (88)
Age	**PSAC[c], n[b] (%)**	4/22 (18)	22/25 (88)
	SAC[d], n[b] (%)	13/48 (27)	48/55 (87)
	Adults, n[b] (%)	2/28 (7)	28/34 (82)
Hematuria	**1+, n[b] (%)**	0/4 (0)	4/4 (100)
	2+, n[b] (%)	1/3 (33)	3/3 (100)
	3+, n[b] (%)	18/78 (23)	78/94 (83)
	None, n[b] (%)	0/13 (0)	13/13 (100)
Maximal egg count	**< 50, n[b] (%)**	2/33 (6)	33/35 (94)
	50-500, n[b] (%)	5/26 (19)	26/32 (81)
	500+, n[b] (%)	12/31 (39)	31/37 (84)
	No eggs, n (%)	0/8 (0)	8/10 (80)
Previous PZQ[e] intake	**Yes, n[b] (%)**	6/41 (15)	41/49 (84)
	No, n[b] (%)	13/57 (23)	57/65 (88)

[a] UT: urinary tract; kidney pelvis dilatation >1cm (if confirmed after bladder voiding or severe), proximal or distal ureter dilatation
[b] distal ureter dilatation was only assessed for 114 patients and kidney pelvis dilatation only for 117, so for any upper tract pathology only for 114 patients complete data is available
[c] Pre-school aged children
[d] School aged children
[e] PZQ: praziquantel

Distal ureter dilatation was assessed in 114 participants with transversal bladder scans at enrolment. Regarding this pathology 98 (86%) scans were rated as interpretable by the expert agreement. Distal ureter dilatation was found in 13 (13%) of the interpretable scans. The longitudinal bladder scan was

performed in 85 participants, 66 (78%) of these scans were rated as interpretable and 7 (11%) of them were found positive for distal ureter dilatation. When combining the results of both transversal and longitudinal bladder scans 100 of 114 (88%) were rated interpretable and 13 (13%) of them as positive for distal ureter dilatation. So there was no additional value of the longitudinal scan in the assessment of distal ureter dilatation. Remarkably, none of the adults had distal ureter dilatation, while among pre-school and school children 18% of scans were found positive for this pathology in each group, respectively.

At enrolment kidney scans were performed in 118 participants. In one participant a mild hydronephrosis could not be confirmed after bladder voiding; this patient was therefore not evaluated for kidney pathology. And 114 (97%) were found interpretable by expert agreement.

The number of cases with kidney pelvis dilatation >1cm at enrolment was 7/114 (6%) with 5 (4%) having unilateral and 2 (2%) having bilateral pathology.

The proximal ureter dilatation was interpretable in 113 of the 118 patients and dilatation was found in seven participants five of them unilateral and two bilateral dilatation.

Detailed data regarding kidney pelvis dilatation is presented in Table 11.

Table 11: Kidney pelvis dilatation >1cm and proximal ureter dilation by experts at enrolment by demography and laboratory

	Kidney pelvis dilatation >1cm		
	Unilateral	Bilateral	All interpretable scans
Total, n (%)	5/114 (4)	2/114 (2)	114/118[a] (97)
By sex:			
Females, n (%)	2/53 (4)	2/53 (4)	53/56 (95)
Males, n (%)	3/61 (5)	0/61 (0)	61/62[b] (98)
By age:			
PSAC[f], n (%)	1/27 (4)	0/27 (0)	27/27 (100)
SAC[g], n (%)	3/55 (5)	2/55 (4)	55/56[b] (98)
Adults, n (%)	1/32 (3)	0/32 (0)	32/35 (91)
By maximal egg count:			
< 50 eggs[c], n (%)	2/36 (6)	0/36 (0)	36/36 (100)
50-500 eggs[c], n (%)	1/31 (3)	0/31 (0)	31/32 (97)
500+ eggs[c], n (%)	2/36 (6)	2/36 (6)	36/37[b] (97)
No eggs, n (%)	0/11 (0)	0/11 (0)	11/13 (85)
Heavy intensity [d], n (%)	3/67 (4)	2/67 (3)	67/69[b] (97)
Previous PZQ[e] treatment:			
Yes, n (%)	2/48 (4)	0/48 (0)	48/50 (96)
No, n (%)	3/66 (5)	2/66 (3)	66 /68[b] (97)
Kidney pelvis and proximal ureter dilatation, n (%)	5/113 (4)	2/113 (2)	113/118 (96)

[a] One participants was not evaluated due to incomplete data
[b] One participant not available
[c] Maximal egg count of *S. haematobium* eggs in microscopy
[d] Maximal egg count of *S. haematobium* eggs in microscopy over 50 eggs
[e] PZQ: praziquantel treatment
[f] Pre-school aged children
[g] School aged children

Figure 8 and Figure 9 show the distribution of US pathologies within the different age, sex and infection intensity groups.

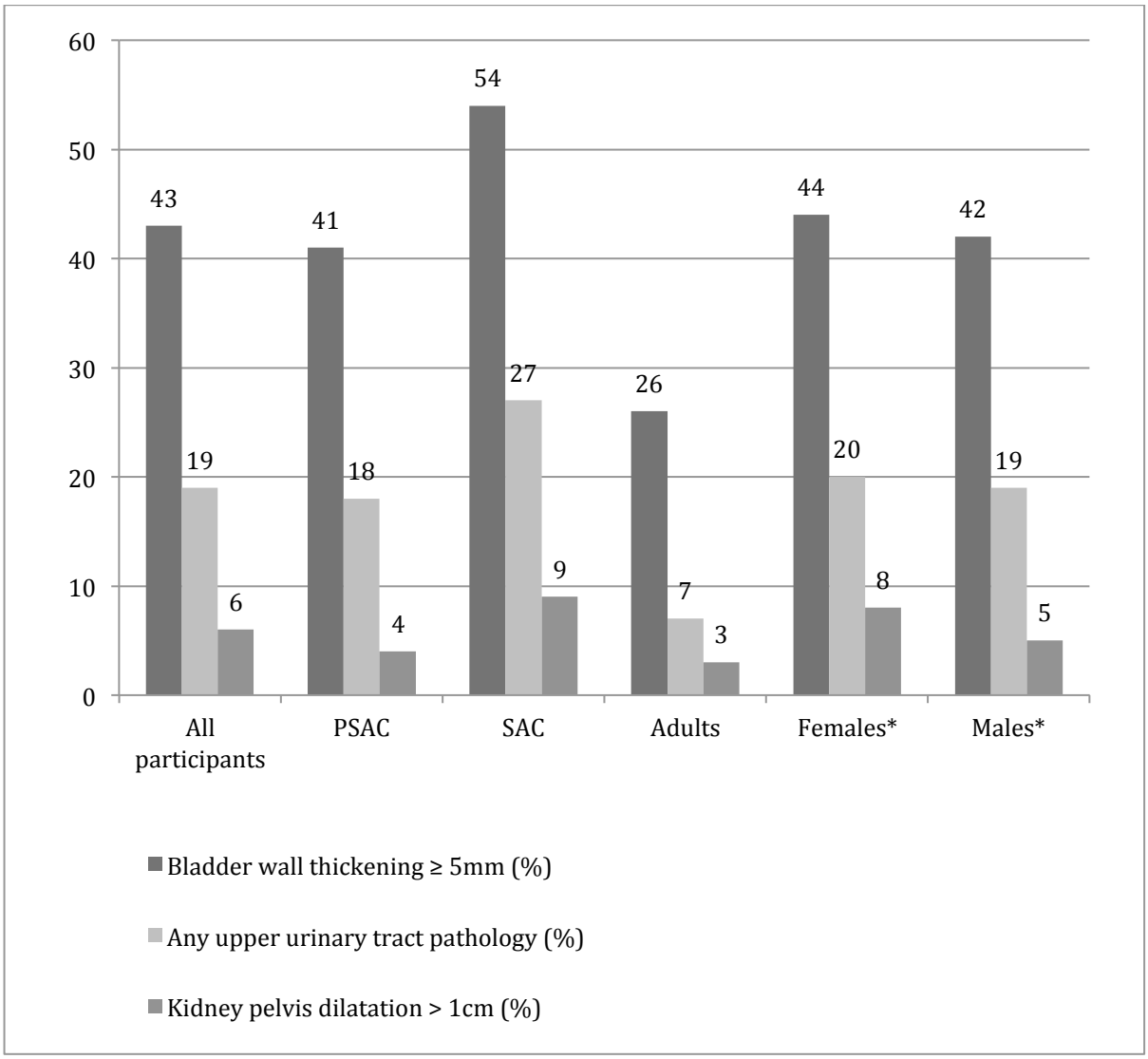

Figure 8: Distribution of US pathologies found by the experts in all interpretable scans subdivided by age and sex; PSAC: pre-school aged children; SAC: school aged children; * n are all interpretable scans of all female/male participants.

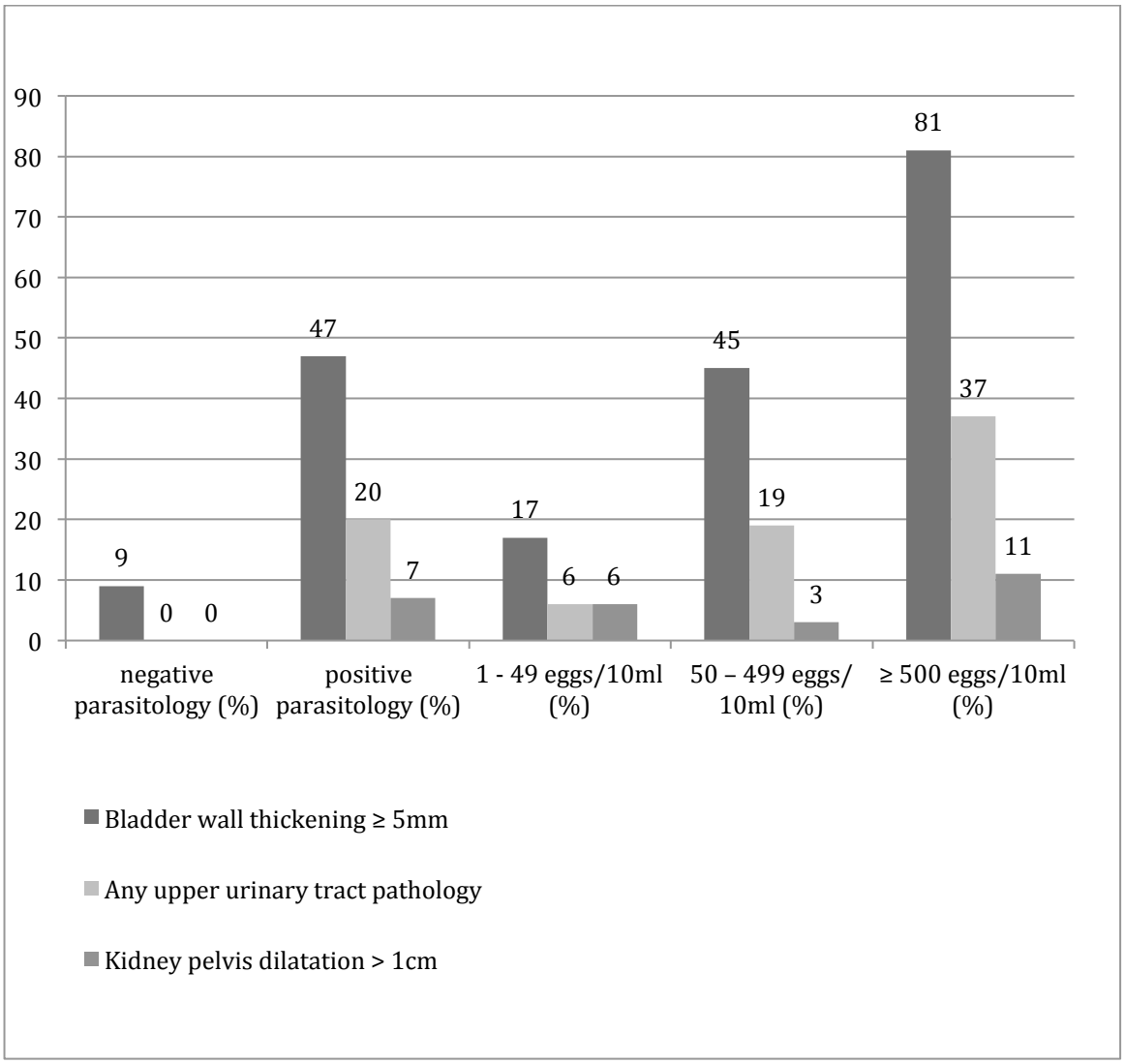

Figure 9: US expert findings according to intensity of infection at enrolment.

The experts detected additional pathologies not related to UGS in 9/118 (8%) patients. Two patients showed non-specific kidney lesions, three patients had free fluid in the pelvis. There was one urachal remnant, one pregnancy related hydronephrosis, one non-specific uterus lesion, and one patient had non-specific strands in the bladder [125].

3.7 Follow up

There were two follow up visits for FASUS participants, M1 and M3. At M1 66 patients were followed up and 82 at M3.

3.7.1 Epidemiology at follow up

The gender and age distribution did not change drastically at follow up visits with 34/70 (49%) and 37/82 (45%) of followed up participants being female at M1 and M3 respectively. The only remarkable difference was that in M3 follow up the proportion of adults dropped from 31% at M1 to 24%.

3.7.2 Clinical urogenital schistosomiasis symptoms

Figure 10 shows the symptom evolution of patients who suffered from hematuria, dysuria or urinary incontinence at enrolment during the follow-up time points M1 and M3.

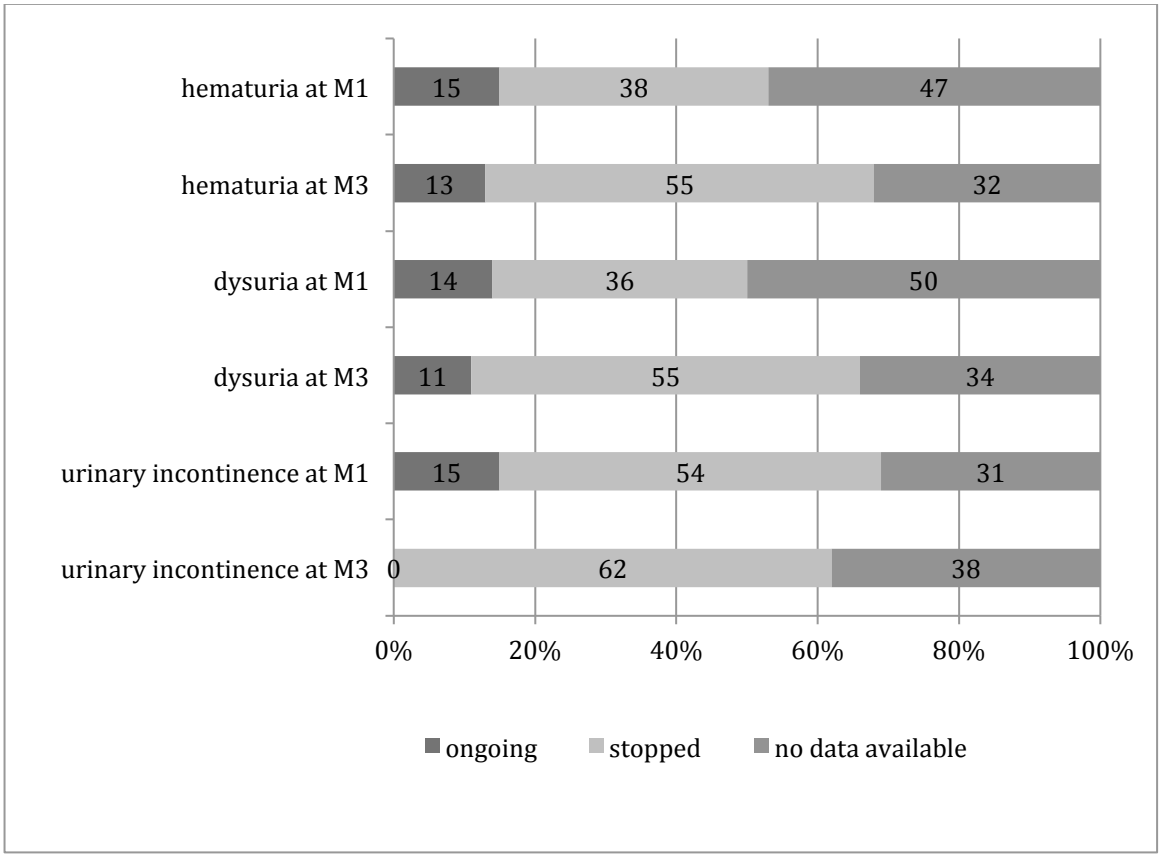

Figure 10: Evolution of reported clinical UGS symptoms after treatment; 100% = all participants positive for the respective symptom at enrolment.

At the follow-up M1, a total of three patients reported vaginal discharge. In two of them this was already present at enrolment while in one it was new.

Genital itching was found in six patients. Half of them had already complained about this symptom at enrolment.

Of initially six participants with testicular swelling, only one reported persistent swelling at M1.

Hematospermia was no longer reported by any of the male patients at M1.

At M3 eight women reported vaginal discharge; in three of them this was a new symptom.

Five complained of genital itching. This was a new occurrence in three cases.

No MGS symptoms were reported at M3.

There were no adverse events reported after PZQ intake at any time point.

3.7.3 Dipstick analysis and parasitological data

During the follow up visits, only two urine samples per participant were targeted for analysis. This was achieved in 77% (54/70) at M1 and 85% (69/81) of cases at M3. Macrohematuria was detected in only two cases at M1 and M3 respectively.

The evolution of hematuria, proteinuria and *S. haematobium* infection at M1 and M3 compared to all individuals who were positive for these parameters at enrolment (100%) is illustrated in Figure 11.

Comparable to enrolment parasitology all urine samples negative for *S. haematobium* eggs in microscopy were also negative in PCR analysis for *S. haematobium* DNA.

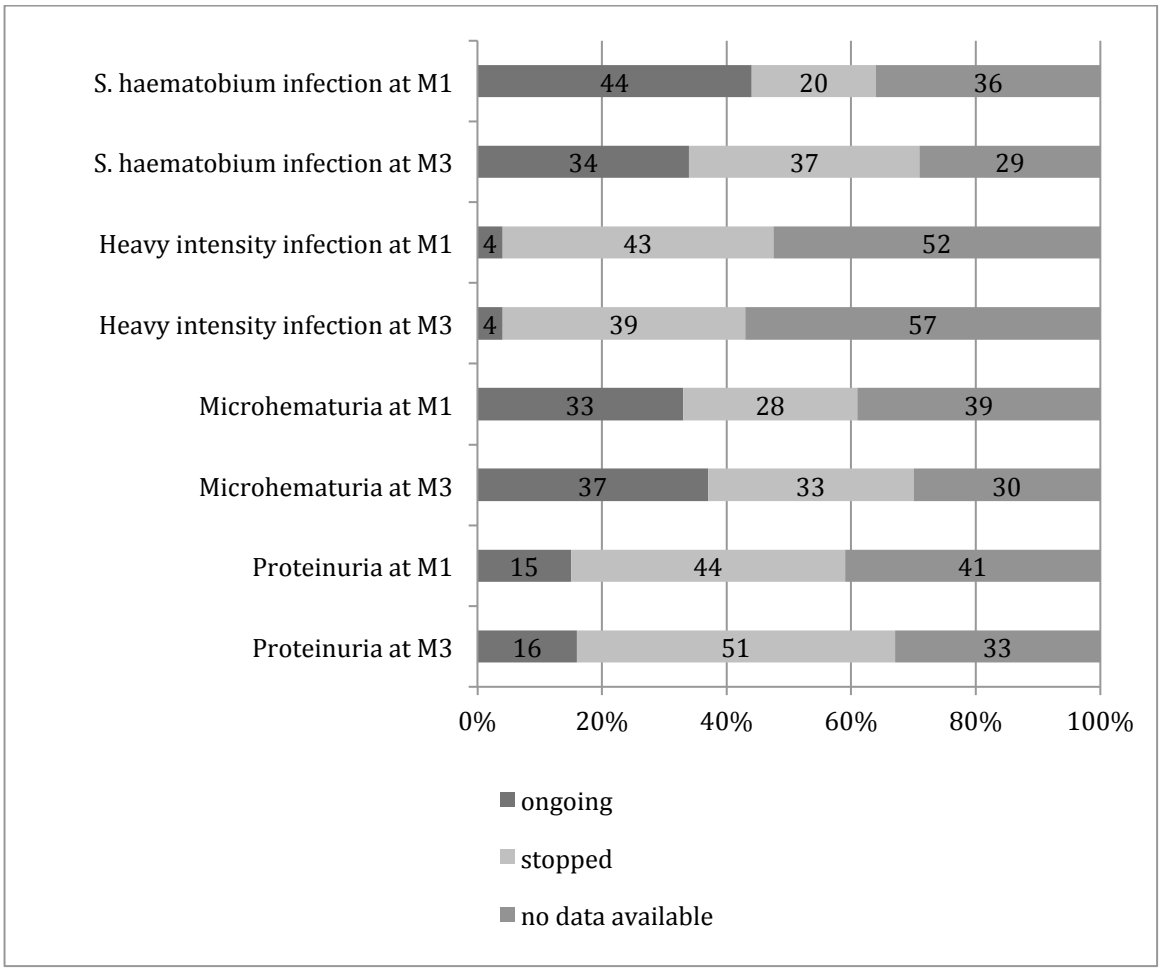

Figure 11: Dipstick analysis and parasitology data at M1 and M3 for individuals positive for these parameters at enrolment (100%).

3.7.4 Expert ultrasound findings at one and three month follow up

As also stated in our paper Remppis et al. [125] at M1 follow up pathologies compatible with UGS were present in 33% (15/46) of scans with sufficient diagnostic quality and in 33% (16/49) at M3 follow up. At M1 17 participants with initially bladder wall thickening ≥ 5 mm at enrolment showed a resolution of this pathology and one former negative participant was now found positive for this pathology. At M3 follow up 23 originally positive participants showed a resolution of the bladder wall pathology and three patients an aggravation.

At M1 there were 6% (3/51) of participants found positive for hydronephrosis. The same applies to M3 (6% (3/54)). At M1 one participant with hydronephrosis at enrolment showed a complete resolution of this pathology and one showed a

partial resolution with a dilated kidney pelvis on only one side. There were no aggravations found at M1. At M3 three participants showed a resolution of the hydronephrosis documented at enrolment two complete and one partial. A newly occurred hydronephrosis was documented in one patient.
Of the three participants who showed persistent renal pelvis dilatation at M3, one no longer excreted any S. haematbium eggs, whereas urine microscopy detected one S. haematobium egg in the other two participants, respectively.
These three participants and their parents were informed about the pathology, given a second dose of PZQ (including the one with negative urine sample), handed out a report form and referred to a specialist in Libreville.

Due to the inconsistent follow up criteria (before and after the amendment) these rates are inappropriate to draw conclusions about treatment success, though.

4 Discussion

The FASUS study was a diagnostic pilot study with 119 symptomatic individuals from an area endemic for UGS in Gabon. This was the first study to evaluate the value of a POCUS protocol for the detection of UGS-related urinary tract pathology as we also state in Remppis et al. [125].

The primary endpoint of the study was to determine the diagnostic accuracy of the FASUS method applied by two inexperienced physicians on-site compared to the gold standard of a remote expert review. With over 88% sufficient image quality and inter-rater agreement of k over 0.6 for bladder wall pathology and over 0.8 for kidney pelvis dilatation between the two operators and the experts, accuracy of this new POCUS application can be rated as good.

In the following, the results are discussed in the context of existing literature and limitations of the study are considered.

4.1 Protocol design and training

Based on the Niamey criteria, pathology compatible with UGS in the FASUS protocol was defined as bladder wall thickening ≥5 mm [80]. As also described in our publication [125] in the course of the study several patients were diagnosed with bladder wall irregularities <5mm, which indicates that even operators with only short and focused US training are able to detect milder bladder wall pathologies via US. In *S. haematobium* endemic areas such findings are likely to be UGS related and earlier detection might be relevant for treatment decisions and prevention of more pronounced pathology. On the other hand differentiation between normal bladder wall and irregularity can be very challenging and a lower cut-off might lead to false positive cases. In order to keep the protocol as simple as possible for the operator, upper urinary tract pathologies in combination with bladder wall pathologies <5mm were not considered as compatible with UGS although they are very likely to be UGS related. This stands in contrast to other studies and should be reconsidered in further and larger evaluation studies [127, 128].

The Niamey protocol comprises only a transverse bladder scan [80]. In order to improve sensitivity, especially for pathologies at the bladder dome, the FASUS team added a longitudinal bladder view. Using the additional view three additional cases of bladder pathology were detected. In our paper Remppis et al. [125] we therefore recommend addition of the longitudinal to the transverse the view for future study protocols and applications.

The FASUS training, which comprised scanning of healthy volunteers with a remote expert feedback and only theoretical pathology detection, proved to be an effective tool to obtain sufficient image quality and inter-rater agreement as also further discussed below. In our paper Remppis et al. [125] we suggest that the training could be optimized using an electronic quiz-like learning module for pathological findings. The optimal time of training, which ranges from hours to several days in other studies, and the number of training scans, still has to be determined [110, 129, 130].

The initial goal of the FASUS team to train local physicians in the FASUS protocol and to establish it in the local structures during the implementation of the study could unfortunately not be fulfilled. Although there was great interest from several local physicians, due to lack of capacity and time resources, participation in the FASUS training did not ultimately succeed. In future studies, however, it is imperative to strive for local physicians to conduct the investigations and train other local physicians in the FASUS method.

4.2 Image quality

The two operators in the field performed 224 US scans in total. Operator 1 did 118 scans at enrolment and operator 2 did 106 scans at M1 and M3 follow up.

As described in Remppis et al. [125] over 90% of bladder scans were assessable for the expert reviewers as well as 99% of right kidney scans, 97% of left kidney scans and proximal ureters, respectively. The lowest percentage of assessable scans was achieved for distal ureter dilatation with 81% assessable longitudinal scans and 88% assessable transverse scans.

These results therefore show, in line with other similar studies before, that good image quality is obtainable even for operators with limited US experience and

only a focused training [131]. Additionally the high ratings on focus, gain and depth demonstrate the effectiveness of the remote training method.

At enrolment the best image quality for detection of bladder wall pathologies was obtained among SAC resulting in 96% assessable scans. As we suggest in Remppis et al. [125] this might be a consequence of a better display of deep anatomic structures and fewer artefacts due to smaller body weight. Among PSAC image quality in bladder wall pathologies was poorest resulting in only 81%, probably due to limited compliance of the very young children during US examination.

Left kidney pelvis and left proximal ureter in adults were also more difficult to assess resulting in only 91% of assessable scans. This might be due to the additional body fat of adult patients and lack of the liver as ultrasound window. All views were more often assessable in male than in female participants, probably due to additional body fat in adult female patients.
There were only three kidney scans and two bladder scans that were rated as “unable to assess” by the operators. This suggests that a potential source of error might be the attempt of the operators to make an assessment despite limitations in image quality [125]. On the other hand, it must also be taken into consideration that the operators on site had considerably more video material at their disposition than the remote reviewers and additionally the data quality was reduced for the expert reviewers due to data compression.
In total, 46 ultrasound scans were performed at the *CERMEL* premises and 178 during home visits. While 11% of home visit scans were rated as unable to asses it was only 7% in the group of scans that was performed in a professional environment. This suggests a tendency that scans performed at patients’ home might have a poorer quality due to external factors like distraction of the patient and operator or unfavourable scanning conditions. Due to different sizes of the two groups these numbers have to be interpreted with caution though.
As described in Remppis et al. [125] sufficient bladder filling was a recurring challenge, which the local investigators were well aware of, but which was sometimes very difficult to achieve on site. In order to keep the protocol as

simple as possible there was no definition of sufficient bladder filling, but the estimation of sufficient bladder filling should be a part of the FASUS training.

4.2.1 Operators learning curve

Operator 1 improved in image quality during the course of the study. He started at 85% assessable bladder scans within the first 59 patients, the share increased to 97% for the remaining. Operator 2 started out with a better image quality with 89% assessable bladder scans within the first 53 scans but didn't show substantial improvement during the course of the study.

The higher starting value of operator 2 might be explained due to the fact that she was able to observe the US scans performed by operator 1 and was even supervised directly by operator 1 during the first few scans.

The more hesitant improvement of operator 2 may be an effect of the lower level of medical education compared to operator 1 or could also indicate that more borderline results at the follow up time points after PZQ treatment were more difficult to assess.

Kidney scans were easier to evaluate and found interpretable by expert agreement in 97-100% for both operators.

While, as described in Remppis et al. [125] sensitivity improved for both operators, there was a slight decline in specificity. Conclusions need to be drawn with caution due to the small sample size, but this might point to a shift from underestimation of pathology to overestimation in both operators with an increasing number of scans.

4.3 Diagnostic accuracy of ultrasound findings

Overall, the markers of diagnostic accuracy were promising. Accuracy in the detection of bladder wall thickening $\geq$5 mm was good with an inter-rater agreement of $\kappa = 0.73$ for operator 1 and $\kappa = 0.64$ for operator 2.

Accuracy in the detection of kidney pelvis dilatation > 1cm was very good with κ over 0.8 (operator 1: $\kappa = 0.85$; operator 2: $\kappa = 0.9$).

The detection of ureter pathology seemed to be more difficult, though. Especially distal ureter thickening, which was missed most of the times by the two operators, had Cohen's kappa scores of 0.19 for operator 1 and 0.27 for operator 2 only. In Remppis et al. [125] we therefore suggest that the examination of the distal ureter should be omitted from the FASUS protocol, considering the difficulty of the assessment and the fact that all cases occurred in combination with bladder pathology and therefore urinary tract pathology was detected anyway.

As also discussed in Remppis et al. [125] the fact that the operators did not find as many pathologies "not related to UGS" as the two experts was not very surprising, since the aim of the FASUS training was just the detection of "pathology related to UGS" and not to provide a thorough US examination.

In a study from Senegal Bonnard et al. [129] performed a study with a similar study design to the FASUS study. A radiologist with experience in applying the Niamey protocol trained another clinician who was unversed in this method. They could show that under direct supervision the learner was able to reach 100% specificity and over 90% concordance after 5 ultrasound sessions (51 examinations).

Compared to the study by Bonnard et al. [129] the results of the FASUS study might seem rather poor. However, the authors themselves appeal to caution when interpreting the high consensus rate towards the end of the study, since apparently especially during the last session predominantly healthy patients were scanned and it was assumed that a normal ultrasound was easier to identify than to detect pathologies accurately.

Other important differences were that the learner was supervised directly by the teacher and both could scan under equal conditions while FASUS operators only received a staggered remote feedback for their training scans and expert reviewers had only cine clips of reduced quality at their disposal. Additionally 79% of FASUS scans were performed at patients' homes, which resulted in poorer quality scans as discussed above and the study used a low budget US device.

The paper by Bonnard et al. [129] also leaves open if the learning clinician was actually naive to ultrasound in general or just to the application of the Niamey protocol.

A study from Janssen et al. [131] investigated the validity of "*focused assessment with sonography for HIV-associated tuberculosis (FASH*)" performed and evaluated by a sonographer on site and a remote review of the scan to diagnose HIV-associated tuberculosis. The remote reviewer also only had a video at his disposal that was transmitted via Skype. Sensitivity and specificity were both 95%. The inter-rater agreement was k = 0.85. These numbers are in line with the FASUS results. The comparability is limited though since local operators partly were also ultrasound specialists and obviously a different POCUS method was performed. The case number was very small with only 14 participants.

Vinayak et al. [132] investigated the use of a POCUS protocol in combination with telemedicine in a different medical context in Kenya. Midwives in rural areas performed an obstetric POCUS. The imagines were transduced via internet and reviewed by a radiologist in order to identify high-risk obstetric patients. The three ultrasound-naive midwives underwent training comprising an e-learning module with a test and 4 weeks of daily lectures and practical training before examining the first patients. Accuracy of scans compared to remote radiologists was 99.63%. Transmission time was short and image quality good.

In view of these studies, POCUS methods appear to be a very promising diagnostic tool that can be used in various medical fields to improve patient care at the point of care. As we state in Remppis et al. [125] the learning via remote review and feedback seems to be feasible and can lead to good accuracy over a longer period of time.

Despite the small case numbers and basic limitations in comparability (different POCUS, different educational levels of trainees) it appears that an intensive training program which exceeds the time frame of the 20 hours FASUS training and which includes directly supervised practical training, could increase the trainees' performance and hence the diagnostic accuracy.

This should be considered in future training concepts of FASUS and should be validated in studies with lager sample sizes.

4.4 Expert ultrasound findings

The expert review of the enrolment scans showed that 43% of the interpretable scans showed bladder pathologies with a wall thickening of ≥ 5 mm and thus were classified as "pathology compatible with UGS". These results are in line with those of other studies with patients in endemic areas although there are also studies that report a significantly higher prevalence of up to 85% bladder wall thickening in randomly selected persons within hyper endemic areas [97, 127, 133]. These high numbers may on the one hand result from a diverging definition according to which "bladder wall irregularity" was already considered as pathologic in some studies. On the other hand, higher infection prevalence among study participants might have been a factor as well as longer exposure times (in contrast to a mean of 3 years of fresh water contact among the FASUS population).

The correlation between increasing intensity of infection and increasing prevalence of bladder wall thickening is well known and was also confirmed by our data [134, 135].

The numbers for “any upper urinary tract pathology” (19% in interpretable scans at enrolment) were also comparable with the results of other studies [97, 127, 136, 137].

Similar to the laboratory results the prevalence of pathologic ultrasound findings did not differ largely between the two sexes with 44% females and 42% males detected with ultrasound findings.

However, again there were noticeable differences between the age groups.

At enrolment 41% of PSAC, 54% of SAC and 26% of adults were found positive for bladder wall thickening ≥ 5 mm.

These numbers are in line with other studies in which bladder pathology was found highest in SAC; namely 43% among PSAC, 67% to 85% among SAC and 24% to 57% among adults (with confirmed *S. haematobium* infection). In these studies, upper urinary tract pathologies also tended to be observed more

frequently in SAC (2-19%) than in PSAC (7%). Although numbers for upper urinary tract pathologies of the FASUS study were small and have to be interpreted with caution, this tendency could also be observed within the FASUS population with five of the seven patients with hydronephrosis being SAC [136, 138, 139]. Older children might be affected more often by severe kidney pathologies because of a longer infection exposure time than younger children with no immunity development yet. The general observation that children are affected more often and have more severe pathologies than adults has also been reported in many other studies and is usually assigned to different fresh water contact patterns and acquired immunity in adults [134, 136, 139]. The comparably high share of PSAC in patients with upper urinary tract pathologies, though, poses the question if MDA in schools reaches infected children early enough or if earlier, community based programs are necessary to prevent serious kidney pathologies.

The prevalence of pathology compatible with UGS was lower in the group of participants that stated to have received PZQ treatment at least once in their lifetime (36% compared to 48% with no previous treatment). For “any upper urinary tract pathology” the difference was also clearly visible with 12% and 20% of upper urinary tract pathology in treated and untreated participants at enrolment, respectively. This supports data from existing literature that one or several treatments in the past may lead to less severe urinary tract pathologies even 10 to 15 years later [96, 97] and underlines the importance of national prevention programs with PZQ treatment.

4.4.1 Follow up ultrasound findings

Resolution of ultrasound pathologies after treatment at M3 was found in 64% of bladder wall thickening ≥ 5 mm, in 38% of participants with any upper urinary tract pathology and in 40% of participants with renal pelvis dilatation over 1 cm. All proximal ureter dilatations and 50% of distal ureter dilatations disappeared.

The rate of bladder pathology resolution after treatment is in line with the existing literature (resolution rates ranging from 50-80% two to 12 months after treatment) [97, 127, 133].

Based on the results of these studies, it can be assumed that the resolution rate in the FASUS population would have increased even further at a later time point.

Both the extensive decrease of ureteral pathologies and the comparably moderate decrease of renal pelvic dilatation could also be observed in previous studies [128, 137, 139]. However, it has also been noted that kidney lesions often require a longer period of time to improve or heal than it is the case with bladder pathologies [97]. This should be considered for determination of follow up time points of following studies or applications of the FASUS method.

Commonly re-treatment is not suggested earlier than 12 month after the initial treatment because pathologies might need at least six month to reduce or resolve [17, 127].

A study from Kenya even showed that prevalence of both hydronephrosis and bladder abnormalities detected by US only showed significant regression after two and three years of PZQ treatment [96]. Therefore, retreatment over time seems to further enhance the regression process.

Due to the lacking possibility of further follow-up visits, though, the patients of the FASUS study with persistent upper urinary tract pathology at M3 received a second dose of PZQ and were referred to a specialist in Libreville for further management. These pathologies would likely have remained undetected and untreated without FASUS. We speculate that FASUS brought an advantage regarding patient management compared to clinical and laboratory assessment alone in these cases. However, it needs to be acknowledged that, despite definition of this issue as a secondary study outcome, the present study design does not allow conclusions on potential advantages of FASUS versus clinical and laboratory assessment alone regarding long term patient outcome. This would require a future study with a head-to-head comparison of application of the FASUS protocol versus clinical and laboratory assessment alone.

As we suggest in Remppis et al. [125] FASUS could be used as a method to identify at-risk patients with more severe findings who need regular follow up

and retreatment. Advanced or therapy-resistant pathologies should be referred to specialists for further diagnostics and management.

4.5 Discussion of epidemiological and clinical data

More than half of the study population (61%) was recruited from an area called Petit Paris 3 (PP3), which is located at the outskirts of Lambaréné right between two small lakes. The inhabitants regularly use these two lakes for bathing and washing their cloth. The situation in the other recruitment regions is comparable with small brooks or lakes that could be identified as potential infection sources. During data collection FASUS participants reported that although the government had set up fresh water pumps for most of the respective areas, these were often perceived as too far away for everyday use. In addition, some individuals expressed a certain mistrust regarding the water quality of these state pumps. The population also seemed only partly aware of the fact that these waters are the source of *S. haematobium* infection. At least in Lambaréné and the surrounding areas these might be reasons why local water bodies are still frequently used for domestic work and bathing. Therefore, the risk of reinfection after treatment is very high among the people who frequent these waters.

The population of the FASUS study was young with a median age of 11.3 years (IQR 6.5-17.5). SAC of 6-15 years represented almost half of the study population (47%). Even though the Gabonese population is comparably young with a median age of 22.7 years the study population does not represent the general composition of the Gabonese population [140]. Presumably, this results from the pre-selection criteria of history of hematuria and the fact that *S. haematobium* infection has its maximum intensity in children around the age of 10; therefore also symptoms like hematuria are likely to be more common and pronounced in this age group than in adults [93]. Secondly, during the recruitment period it could be observed that parents tended to let their children participate in the study in order to receive free health care, but often did not want to enrol themselves.

Only 43% of all study participants stated that they had received anti-schistosomal treatment at least once in their life, which indicates poor access to health care for most of the study population and absence of an effective program of preventive chemotherapy. In the subgroup of SAC only 32% had received PZQ treatment within the last 12 month. This fits the WHO information about Gabon that national preventive chemotherapy programs for SAC as recommended by the WHO only started in 2016 which led to coverage of 37% of SAC [118].

Symptoms of FGS were very common upon the female FASUS study population of all ages. Genital itching was found in 52% of female participants and in the age groups of PSAC and adults even over 60% reported this symptom; malodourous vaginal discharge was reported by 22% of female patients and urinary incontinence by 26%. Although these symptoms are not very specific and can also be caused by other diseases such as yeast infections, they also match other reports on 30-50% of women reporting symptoms of FGS in *S. haematobium* endemic areas [34]. Especially the high number of FGS symptoms among children is alarming given the possible complications if left untreated (decreased fertility, ectopic pregnancies, increased susceptibility to HIV-infection) [41].

Symptoms of MGS were less common in the FASUS population. Only 11% of all male patients reported testicular swelling and only one participant reported hematospermia. However, due to the age composition of the study population with a large proportion of pre-puberty participants, there was no data collected on hematospermia for 82% of male participants. Therefore data might not represent the actual prevalence of MGS in Gabon.

Recurrent UTIs were reported not very common among the study population. Only two of the 118 participants reported recurrent UTIs. Compared to other studies that found a prevalence of UTIs ranging from 30% to 80% among patients with UGS, this number is surprisingly small and might be a consequence of under-diagnosis of this pathology since dysuria was very common [141]. This hypothesis is also supported by the fact that proteinuria

was found in 74% of FASUS participants in urinary dipstick analysis, leukocyturia in 73% and several samples were found positive for nitrit.

4.5.1 *Schistosoma haematobium* infection rates

All urine samples were analysed with dipstick tests and subsequently examined by parasitological microscopy for *S. haematobium* eggs. In dipstick analysis 87% of participants were found positive for microhematuria, over 90% of them with the highest intensity of 3+. Combined with an overall prevalence of 89% of *S. haematobium* positive FASUS participants, it can be assumed that schistosomiasis is the major cause of hematuria in patients in *S. haematobium* endemic areas of Gabon. The FASUS data also supports other studies in their findings that both, reported macrohematuria and microhematuria in dipstick analysis, are a sufficient screening parameter for *S. haematobium* infection in endemic regions [127, 142, 143].

Reported prevalence of *S. haematobium* infection in the population around Lambaréné ranged from 15% to 40% in other studies from this region. Prevalence was very dependent on proximity and use of infested water sources [144-146]. Due to the pre-selection of the study population by the symptom of hematuria in this study, data are not comparable.

Considering the occurrence of *S. haematobium* infections in different population subgroups, it appears that there were no significant differences in infection prevalence between the two sexes but clear differences between the different age groups. While the age group with the highest overall prevalence (95%) were SAC, high intensity infection was most common (79%) in children under six years of age. This differs from the literature, where both infection and intensity peak is usually reported between 10 and 12 years [93, 138, 147]. This high percentage of heavy intensity infection in pre-school age children might be biased by the fact that parents only made their young children participate in the study if symptoms were severe and therefore higher egg excretion might have been more likely. However, it still outlines the necessity of finding new preventive chemotherapy concepts which also cover this age group, since the preventive treatment programs in schools as recommended by the WHO do not

reach these children [148]. This might be achieved with family or community based treatment programs.

The comparatively low number of only 50% of infected adults suffering from heavy intensity infection is in line with other reports in the literature and probably is a consequence of an acquired immunity as a result of several infections over lifetime [92-94].

4.6 Additional urine samples, collection time and examination by PCR

In order to increase the sensitivity of urine microscopy several urine samples were collected at every time point (three at enrolment and two at M1 and M3). This increased the detection rate with five, eight and seven participants additionally found positive by the supplementary samples at enrolment, M1 and M3, respectively. The benefit of this method was also supported by the fact that none of the 13 all-negative cases was found positive for *S. haematobium* DNA in PCR analysis, thus there were no false negatives which means that no positive patients were missed by the parasitological analysis.

In the literature it is widely accepted that the daytime of highest urine egg excretion is from 10 am to 2 pm [123]. Of FASUS urine samples 61% were successfully collected during this time slot, due to organizational reasons however 32% of samples were collected before 10 am. As suggested the percentage of positive samples was higher in the noon group than it was in the morning group (69% noon vs. 54% morning). This data should be interpreted with caution though, as the different sizes of the two groups may have biased the results.

In contrast to our expectations, the additional examination of samples, found negative in urine microscopy, by PCR, did not detect any false negative samples. This comparatively expensive and labor-intensive method therefore does not seem to offer any additional benefit to the much easier-to-implement microscopic urine examination. This seems to be true at least in endemic areas where high egg excretion is expected.

4.7 Limitations

As stated in Remppis et al. [125] the reference of a remote expert review and only remote feedback during the training period is inferior to a locally performed review and direct supervision of an expert on site. Due to the absence of local experts, this was the only available option, though.

Another limitation was image quality. Both, image quality for the local operators due to the low quality US device and for the experts due to data transmission as cine clips, may have limited diagnostic performances. However, the performance of US scans with low-quality devices is a good simulation of the conditions in the field, where FASUS might be applied in future. These limitations as well were inevitable due to the lack of better alternatives.

4.7.1 Protocol

An important limitation of the FASUS study was the incomplete follow up data. Ideally all participants regardless of their infection or US pathology status should have been followed up to collect a complete data set to make the three time points directly comparable. This limitation is mainly a consequence of the fact that FASUS was a pilot study and no other comparable study was available for orientation in structure and implementation. The protocol was changed within the course of the study and therefore the follow up conditions changed for all participants who were recruited after the amendment. Thus, number and composition of the study population was different at every time point, which makes the comparison of the collected data more complex.

Additionally the questionnaire to evaluate the US scans was also adapted a few times to optimize data collection and the longitudinal bladder scan was added after 27 patient scans had already been completed. This led to an incomplete dataset for some variables such as sufficient bladder filling, ureter dilatation and longitudinal bladder view.

4.7.2 Detection Bias

When local operators did not suspect pathology, they only sent one clip of every organ view to the expert reviewers. If pathology was suspected still imagines

were added. Due to this practice expert reviewers already knew if local operators did suspect pathology or not. This might have biased the expert reviewers and led to a higher probability of experts to agree with the operators.
In future studies with a telemedicine-based design the use of a standardized set of video clips for every patient regardless of the operators' findings might be better to avoid this kind of bias.
Secondly, the knowledge about the presence of pathology at enrolment might have biased both operators and experts in the interpretation of follow up US exams especially in the case of borderline pathologies.
Another variable that is hard to asses and might have influenced the results of the study is the fact that prevalence of UGS-related pathology might differ in the group of US scans with insufficient image quality that could not be assessed compared to the assessed images.

4.7.3 Operator dependency

Another major limitation of the transferability of the study data to other scenarios is the operator-dependence inherent to ultrasound. However, point-of-care ultrasound protocols reduce operator-dependency by a clearly defined protocol including standardized views and pathology definitions.
Both, urine microscopy and reading of the dipstick colour scale, are operator dependent too to a certain extend. These factors are inevitable limitations, though. They were attempted to be minimized by the fact that several samples were collected for every patient and several scientific staff members worked in the parasitology laboratory all of them performing all the required work steps defined in standard operating procedures.

4.7.4 Composition and selection bias

Since the recruitment was done via convenience sampling the rate of infection and UGS-related pathologies among the study participants do not reflect the prevalence among the general Gabonese population.

By including only symptomatic patients with suspected *S. haematobium* infection the study population is not representative for the general population in Gabon or even endemic regions. Since normal anatomy might be easier to identify by US and on the other hand borderline findings in patients with light infections might be harder to identify, actual FASUS sensitivity and specificity might differ from the results of this study when performed in an unselected population. The age composition of the FASUS population might also have biased the results since the largest share were SAC who are generally easiest to assess with ultrasound. Further investigations in a larger number of unselected participants are necessary for a generalizability of the findings.

4.8 Conclusions

Considering the on going high prevalence of *S. haematobium* infection and in several regions still deficient prevention programs, the use of ultrasound as a diagnostic tool to detect urinary tract pathologies could be very useful to survey the prevalence of morbidity and regression of urinary tract pathologies after treatment and detect severe pathologies that need special management.

As stated in Remppis et al. [125] this study showed that FASUS is a promising method with the potential to provide an easy-to-learn bedside method for less experienced local clinicians to detect UGS-related morbidity for symptomatic patients in endemic regions. The presented FASUS concept comprising short and focused training and a remote review seems to be feasible and leads to a good overall diagnostic accuracy.

If following studies validate the data and confirm the utility of FASUS as a diagnostic tool, international standards for training and application algorithms should be established.

Suggestions for future studies include larger and unselected study population to reduce effects of pre-test-selection on sensitivity and specificity, local physicians as operators in order to adapt training to local knowledge and skills, and direct expert supervision in order to further improve training and diagnostic accuracy.

5 Summary

Schistosomiasis is a neglected tropical disease. In UGS inflammation and fibrosis of the urinary tract is leading to bladder wall thickening and hydronephrosis in infected individuals.

This thesis reports the development of "*Focused assessment with sonography for urinary schistosomiasis (FASUS)*" for evaluating bladder, kidneys and ureters for pathological changes due to UGS as also reported by Remppis et al. [125].

In a diagnostic pilot study from 2015 to 2016 FASUS was developed and applied by one early career physician and a medical student from Germany in a *S. haematobium* endemic area in Gabon. Following a standardized, focused training period the two operators applied the FASUS protocol on 118 symptomatic participants and collected demographic, clinical and parasitological data. Recorded ultrasound clips were sent to two ultrasound experts in Europe for review and serving as a diagnostic reference.

Sufficient image quality for remote evaluation was achieved in 90% of bladder scans and 97% of kidney views. The agreement rate between the investigator on site and the remote review for bladder wall thickening was good for both operators with a Cohen's kappa value of 0.73 for operator 1 and 0.64 for operator 2. For kidney pelvis dilatation inter-rater agreement was very good with Cohen's kappa 0.85 for operator 1 and 0.9 for operator 2. Pathology compatible with UGS at enrolment was found in 43% (51/118) of patients by the two operators on site and in 43% (46/107) by the two expert reviewers among scans of sufficient image quality. Three months after PZQ treatment over 60% of patients showed a sonographic decrease in UGS related pathology.

These data show that the FASUS protocol is a very promising, easy-to-learn tool, which can be applied by ultrasound-naïve operators in *S. haematobium* endemic regions and thus offers the possibility to examine populations at risk in order to identify UGS related pathologies.

Larger validation studies are needed to verify the results and establish international standards for training and application algorithms.

6 Summary in German language

Schistosomiasis gehört zu den vernachlässigten Tropenkrankheiten (Neglected Tropical Diseases, NTD) und kann bei infizierten Personen Inflammation und fibrotische Veränderungen der Harnwege verursachen, die zu Blasenwandverdickung und Hydronephrose führen.

Diese Doktorarbeit beschreibt, wie bereits von Remppis et al. berichtet, die Entwicklung von "*Focused assessment with sonography for urinary schistosomiasis (FASUS*)" zur Beurteilung von Blase, Nieren und Harnleitern, um pathologische Veränderungen durch urogenitale Schistosomiasis zu diagnostizieren [125].

In einer diagnostischen Pilotstudie von 2015 bis 2016 wurde FASUS entwickelt und von einem jungen Arzt und einer Medizinstudentin aus Deutschland in einem *S. haematobium* Endemiegebiet in Gabun angewendet. Nach einem standardisierten, fokussierten Training wandten die beiden Anwender das FASUS-Protokoll bei 118 symptomatischen Teilnehmern an und sammelten zusätzlich demographische, klinische und parasitologische Daten. Aufgezeichnete Ultraschall-Clips wurden an zwei Ultraschall-Expertinnen in Europa zur Begutachtung geschickt welche als diagnostische Referenz diente. Eine ausreichende Bildqualität für die Fernauswertung wurde in 90% der Blasenaufnahmen und 97% der Nierenaufnahmen erreicht. Die Übereinstimmungsrate zwischen dem Untersucher vor Ort und dem Fern-Review für Blasenwandverdickung war für beide Anwender gut, mit einem Cohen-Kappa-Wert von 0,73 für Operator 1 und 0,64 für Operator 2. Bei der Nierenbeckenerweiterung war sie mit einem Cohen-Kappa-Wert von 0,85 für Operator 1 und 0,9 für Operator 2 sehr gut. Mit UGS zu vereinbarende Pathologien wurden zu Beginn der Studie bei 43% (51/118) der Patienten von den beiden Anwendern vor Ort diagnostiziert. Die beiden Expertinnen fanden ebenfalls bei 43% (46/107) aller Ultraschalluntersuchungen mit ausreichender Bildqualität, mit UGS zu vereinbarende Pathologien. Drei Monate nach der Behandlung mit PZQ zeigten über 60% der Patienten sonographisch einen Rückgang der pathologischen Veränderungen.

Diese Daten zeigen, dass FASUS eine vielversprechende, leicht zu erlernende Methode ist, die von ultraschallunerfahrenen Anwendern in *S. haematobium* Endemiegebieten angewandt werden kann und somit die Möglichkeit bietet, Risikopopulationen zu untersuchen, um Pathologien zu identifizieren.

Es müssen größere Validierungsstudien durchgeführt werden, um die Ergebnisse zu verifizieren und internationale Standards für Ausbildung und Anwendungsalgorithmen zu etablieren.

7 References

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8 Publication

Parts of the data of this thesis have already been published in the following paper:

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Erklärung zum Eigenanteil der Dissertationsschrift

Die Arbeit wurde im Institut für Tropenmedizin, Reisemedizin und Humanparasitologie am Universitätsklinikum Tübingen unter Betreuung von Prof. Dr. Peter G. Kremsner (*Institutsdirektor*) durchgeführt.

Die Konzeption der Studie erfolgte durch J. Remppis (*Abteilung für Hämatologie und Onkologie, Klinik für Kinder und Jugendmedizin, Universitätsklinikum Tübingen*), A. L. Bustinduy (*Department of Clinical Research, London School of Hygiene and Tropical Medicine; Centre de Recherches Médicales de Lambaréné (CERMEL*)), T. Heller (*Lighthouse Clinic, Kamuzu Central Hospital*), E. Joekes (*Liverpool School of Tropical Medicine; Department of Radiology, Royal Liverpool University Hospital NHS Trust*) und S. Bélard (*Klinik für Pädiatrie m.S. Pneumologie, Immunologie und Intensivmedizin, Charité - Universitätsmedizin Berlin; Centre de Recherches Médicales de Lambaréné (CERMEL); Berlin Institute of Health*).

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Sämtliche Ultraschalluntersuchungen wurden von J. Remppis oder mir selbst vorgenommen; die Experten-Reviews wurden von E. Jokes und S. Bélard durchgeführt. Die Laboruntersuchungen (Urinfiltration und Mikroskopie) wurden durch N. García-Tardón (*Centre de Recherches Médicales de Lambaréné*

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Ich versichere, das Manuskript selbständig (nach Anleitung durch Prof. Kremsner, J. Remppis und S. Bélard) verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Hamburg, den

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