Published in final edited form as:
Parasite Immunol. 2013 September ; 35(0): 295–301. doi:10.1111/pim.12040.

The use of imaging to detect schistosomes and diagnose schistosomiasis

Patrick Skelly, PhD
Molecular Helminthology Laboratory, Department of Infectious Disease and Global Health, Tufts Cummings School of Veterinary Medicine, 200 Westboro Road, North Grafton, MA 01536

Patrick Skelly: Patrick.Skelly@Tufts.edu

Summary

Several imaging modalities have been employed to examine schistosomes and monitor schistosome-induced pathology. Ultrasound is a non-invasive imaging method that has long been used in the laboratory and in the field to evaluate pathological changes, notably fibrosis, that arise as a consequence of the host response to schistosome eggs lodging in a variety of tissues. Ultrasonography has been widely used to monitor changes in the extent of fibrosis and in spleen/liver enlargement following chemotherapeutic treatment for schistosomiasis. Imaging methods to monitor schistosomes themselves *in vivo* (as opposed to detecting schistosome-induced pathology) include positron emission tomography (PET) and fluorescence molecular tomography (FMT). Both approaches rely on schistosome uptake of tracers that are introduced into infected animals and that can be detected externally. These methods have been used to successfully detect schistosomes *in vivo* and to monitor their elimination following chemotherapeutic treatment. Direct monitoring of live schistosomes *in vivo* has been achieved using intravital microscopy (IVM), when the infected tissues of anaesthetized animals are exposed. Finally, schistosome eggs have been visualized by confocal laser scanning microscopy in infected mice as well as in a human patient with schistosomiasis hematobium. Further advances in imaging technologies seem likely to provide greater insight into disease progression and into the biology of schistosomes in the most relevant setting – within a live animal.

Keywords

schistosoma; ultrasound; fluorescence molecular tomography (FMT); positron emmision tomography (PET); intravital microscopy (IVM)

Schistosomes are intravascular parasitic platyhelminths (blood flukes) that infect over 200 million people around the world (1). The worms can cause the disease schistosomiasis which remains one of the most prevalent parasitic diseases in developing countries and has substantial economic and public health impact (2). Three schistosome species are of major importance for humans; these are *Schistosoma mansoni*, *S. japonicum* and *S. hematobium*. Infection with these parasites follows direct skin contact with fresh water that contains free-swimming larval forms known as cercariae. These larvae can actively penetrate the skin in minutes (3). Once inside, the parasites undergo a complex morphological and biochemical change as they adapt to the change from fresh water to the mammalian body; they lose their bifurcated tail and slough off their outer tegument, replacing it with a new, double lipid-bilayer (or heptalaminate) outer covering (4). Transformed cercariae are called schistosomula and these migrate through the dermis and invade the circulatory system. The parasites are carried in the blood through the heart to the lungs and arrive in the vasculature of the liver where they mature (5). In contrast to other trematodes, schistosomes have separate sexes and it is in the blood vessels of the liver that the male and female parasites

pair. Next, the worm couples migrate against the flow of blood to their preferred egg laying sites. The precise final destination differs for different schistosome species; the posterior branches of the superior and inferior mesenteric veins are preferred by *S. mansoni*, the anterior branches of the superior mesenteric veins in the case of *S. japonicum*, or the vesical plexus and veins draining the ureters in the case of *S. haematobium* (5). Egg production begins several weeks after infection and continues for the life of the worm. Eggs pass from the lumen of the blood vessels into adjacent tissues, and many then pass through the intestinal or bladder mucosa and are shed in the feces (in the case of *S. mansoni* and *S. japonicum)* or in the urine (in the case of *S. haematobium*) (6). The life cycle continues when the eggs hatch in fresh water and motile, ciliated larval forms (called miracidia) are released. These can infect select species of freshwater snails. Asexual replication within the snail through two generations — called primary and then daughter sporocysts — leads to the generation of large number of cercariae. These are released and, upon infecting a final, mammalian host, complete the life cycle.

Schistosome pathology

Imaging modalities have been used to diagnose and monitor schistosome infection in humans and experimentally infected animals. Examining the specific pathological changes caused by the different schistosome species using imaging has been informative. These pathologies arise not, for the most part, as a direct response to adult schistosomes alive in the veins since these do not appear to cause notable inflammation or to impede hemostasis (7, 8). Instead it is parasite eggs, released in the hundreds every day that are the main cause of the problem (9). In contrast to the adult worms, parasite eggs elicit a strong inflammatory reaction resulting in the egg being surrounded by a variety of immune cells to form a unit called a granuloma (reviewed in (10)). Both T and B lymphocytes, macrophages, giant cells, mast cells, plasma cells, fibroblasts and eosinophils can all contribute to the granuloma (11). With time, a fibrotic reaction predominates and the schistosome embryo at the core of the granuloma dies (12). Tissues in which schistosome eggs are found therefore become highly fibrotic. For *S. mansoni* and *S. japonicum* the alimentary tract, primarily the large bowel, develops fibrosis. This is the case because the mature parasites in the mesenteric veins discharge hundreds of eggs per day through the mucosa and it is here that a fibrotic reaction develops (13). Some eggs deposited within the bowel wall may be no longer able to migrate because they have been trapped. In contrast, for *S. hematobium* which live as adults largely in the vesicle plexus, inflammatory changes will principally surround eggs in the ureters and bladder (14). Granulomas caused by *S. haematobium* have additionally been seen in the testes and in the female genitalia, ovary, cervix, and endometrium (15).

Not all the eggs shed by the female worms get trapped in the large bowel or in the bladder and ureters. Many are passed to the outside in feces and urine to perpetuate the life cycle of the parasite. However, large numbers can flow back into the portal veins from mesenteric venules. Here they can lodge in the walls of the small portal veins and in the periportal connective tissue, where they elicit inflammation, leading to multiple granulomas in the liver followed by fibrous tissue formation (1). Such lesions can obstruct the small portal veins before they enter the hepatic sinusoids, causing a presinusoidal pattern of liver damage, known as Symmers' fibrosis (16–18). Initially, the fibrous tissue is confined to the portal tracts, with liver cells being uninvolved. Lesions in the liver can lead to portal hypertension and its most important complication, bleeding from dilated veins in the esophagus (esophageal varices) (1).

Schistosome ova have also been detected in other organs, notable the lungs (15). Portal hypertension and collateral circulation may be important to shunt eggs to this site. *S. haematobium* eggs are found more frequently in the lungs compared to those of other

schistosome species (19). Granuloma formation can lead to diffuse fibrosis particularly in the peribronchial regions. There may be some damage to the lung air sacs manifest as mild emphysema (19).

Diagnostic imaging using ultrasonography

The pathological changes described above that are brought about following schistosome infection can often be detected using a diagnostic imaging technique called ultrasonography. Here sound pressure waves whose frequency is greater than the upper limit of the human hearing range (ultrasound) are used to visualize subcutaneous body structures. Using a probe, an ultrasound machine transmits high-frequency (1 – 5 megahertz) sound pulses into the body. Sound waves hit a boundary between internal tissues and some get reflected back to the probe. By calculating the distances and intensities of the sound echoes from internal tissue boundaries, an ultrasound machine can then display a two dimensional image of tissue morphology. The technique is noninvasive, radiation free and inexpensive, and has, for over 50 years, become a key method to assess the morbidity associated with schistosomiasis (reviewed in (20) and (21)). In addition, since ultrasonography provides a direct image of the pathologic change, is relatively simple to perform and is reported to be well accepted by communities, it can be readily employed in field-based studies (22, 23). Limitations of ultrasonography include its high dependence on operator skills and the fact that inter-observer agreement can be low (24).

International expert committees, convened by the World Health Organization (WHO) met on a number of occasions to attempt to objectively define and categorize the pathologic changes associated with schistosomiasis as defined by ultrasonography and to standardize the different scoring systems used in different areas. These meetings led to the development of consensus WHO-recommended ultrasonography protocols (25). The severity of disease in schistosomiasis can be measured by ultrasonography based on these WHO criteria.

Ultrasonography analysis of hepatic disease includes an assessment of the outline of the liver, specific measurements of the size of the left and right lobes of the liver; and measurement of the inner diameter of the portal vein (26). Among the earliest evidence of infection, especially with *S. mansoni* and *S. japonicum* as assessed by ultrasonography is loss of the normal smooth liver outline and/or thickening around the portal vein and its main branches. These areas are considered highly echogenic (i.e. they have an enhanced ability to return an ultrasound signal), especially around the porta hepatis. There may be enlargement of the splenic veins also and, when portal hypertension develops, there is usually splenomegaly (27). Periportal fibrosis can be assessed qualitatively by image classification (28) or quantitatively by determining the diameter of secondary portal branches (29). Infection with *S. japonicum* can lead to the development of a network pattern of parenchymal fibrosis that has been described as fish scale-like.

It is recommended that ultrasonography measurements be established within each new locality as there can be much geographical variation (30); the WHO protocol states that measurements of organ size and vein diameter should be height-adjusted, using standard reference measurements for healthy members of the same population (25). *S. japonicum* can cause more damage to tissues and organs than the other species because the female worm has a considerably higher egg output; in addition, the smaller size of the eggs of *S. japonicum* may permit them to enter smaller portal vessels and so impact more of the liver (20).

Ultrasonography is also a good way of detecting hypertrophy (enlargement) of the bladder mucosa, thickening of the bladder wall, and bladder calcification that can be associated with *S. hematobium* infection. Impedance in the flow of urine from the kidney can lead to an

accumulation of urine in the kidney (a condition known as hydronephrosis) and this is usually apparent using ultrasound also. Because there is a strong association between schistosomiasis haematobium and squamous cell carcinoma of the bladder (31, 32), ultrasonography to accurately record bladder wall thickening can be particularly important. Successful treatment for schistosomiasis should lead to bladder lesion regression over several months, as assessed by repeat ultrasonography. If a bladder lesion does not improve, long-term follow-up is required to exclude malignancy. While the bladder is usually the focus of ultrasound analysis in *S. hematobium* patients, considerable alterations in liver ultrasound image patterns can also be observed in these individuals (33). This is because, in addition to being detected in the vasculature of the bladder, *S. hematobium* worms can also be found in the mesenteric and portal veins of individuals examined at necropsy and many *S. hematobium* eggs can be found in their livers (15).

In 1992 ultrasonography was employed to assess periportal fibrosis and liver and spleen size in a large study in an area of high *S. japonicum* transmission in China (30). It was reported that the age-prevalence of infection (as assessed by fetal egg-counts) was similar to that reported for *S. mansoni* and *S. haematobium*, maximal in the 10–14-year-old group. However, the highest rate of pathology detected by ultrasonography was in subjects older than 30 years (30). This is not surprising given the progressive nature of the disease.

In 2006 abdominal ultrasonography was performed on 2,247 school children and urinary tract ultrasonography on 2,822 children from 29 schools in Mali following WHO protocols. This approach was reported to be most useful in detecting *S. haematobium* pathology but tended to overestimate the risk of portal vein dilatation and left liver lobe enlargement associated with *S. mansoni*. Nonetheless the authors encourage the cautious inclusion of ultrasonography to monitor control interventions (34). Indeed, ultrasonography has been often used to monitor regression of clinical symptoms, liver and spleen size after treatment in endemic areas (reviewed in (20)). Documented ultrasonographic regression of liver fibrosis was reported in 28 patients three years after treatment with the anti-schistosome drug praziquantel (PZQ) (35). Additionally, in 84 patients treated with the drug oxamniquine, regression of fibrosis in 32%, and the disappearance of splenomegaly in 48%, was reported after four years (36). In another study, ~50 patients with different grades of pathology were examined every 3 months for 1.5 years after treatment with PZQ (37). While regression of mild to moderate fibrotic changes and of splenomegaly was seen, this was not the case for advanced hepatic fibrosis or for the formation of collateral veins of the portal tract. It was concluded that the damage to the portal system may be irreversible when the intensity and duration of the infection have progressed beyond a critical point (37). These findings have been confirmed in a more recent study in which 578 people in Southwest China were followed for up to five years; it was found that *Schistosoma japonicum* fibrosis declined significantly following treatment as assessed using ultrasound but detectable improvements were limited among individuals with advanced fibrosis (38).

Imaging hepatic function

Recently an imaging modality, employing a glycol-derivative (6-3′,6′-Diaza-5′-oxo-3′-(2′-triphenymethylthioethyl)-8′-triphenyl-methylthio]octanamidohexyl b-Nacetyl-galactosamine) abbreviated OCTAM, was used to image hepatic function in schistosome-infected mice (39). The affinity of radiolabeled OCTAM for hepatocytes permitted *in vivo* imaging of the liver, using single photon emission computed tomography. Following labeled OCTAM administration to mice infected for different time periods, serial images were obtained. These showed a greater delay in label uptake in the liver with prolonged infection. In addition, the retention of label was inversely correlated with stage and grade of schistosome infection. This suggested that accumulating liver injury caused by schistosomiasis impaired label uptake and retention and it was proposed that the imaging

technique might be suitable for hepatic function evaluation following experimental schistosome infection (39).

Detecting schistosomes *in vivo* using positron emission tomography (PET)

Imaging via ultrasonography in humans and imaging following OCTAM administration in mice bothdetect pathological outcomes of schistosome infection. Other imaging modalities have been more recently employed to directly detect the parasites themselves *in vivo*. One such technology is positron emission tomography (PET) which is an imaging technique whereby gamma rays, derived from a radionuclide tracer that has been introduced into the body, are detected externally. Three dimensional images of tracer concentration are then generated by computer analysis. Detecting schistosomes in the blood stream by PET exploits the fact that the intravascular parasites are avid consumers of glucose; adults have been calculated to metabolize their dry weight in glucose every five hours (40). Glucose is taken up by facilitated diffusion across the tegument (skin) of the intravascular life-stage worms via a series of glucose transporter proteins located in the tegumental membranes (41–44). This strong avidity of schistosomes for glucose has been used in attempts to detect the parasites living within infected animals using PET. This is because an analog of glucose known as FDG (2-deoxy-2[^{18}F]fluoro-D-glucose) is commonly used as a biologically active radionuclide tracer in PET (45). In 2010 researchers set out to determine if the high metabolic demand of intravascular schistosomes for glucose results in their accumulating sufficient quantities of FDG to permit *in vivo* imaging of the parasites using PET (45). It was first demonstrated that, *in vitro*, adult worms can import FDG at levels suitable for PET. Next, athymic nude mice were infected with *S. mansoni* cercariae and ~6 weeks later the mice were subjected to periorbital injection of FDG in saline and subjected to PET.

Intense radioactive signals were observed in the heart, kidneys, bladder and regions of the lower abdomen in control and infected mice and high background uptake was seen also in the colon and small intestines (45). Three-dimensional “regions of interest” (ROIs) were drawn around the common portal vein, inferior to the liver and in areas of increased label uptake in the liver. A positive linear correlation was reported between the total radioactivity within ROIs and the number of worms later recovered by perfusion (45). FDG tracer uptake showed a stronger positive correlation with worm burden when more than 50 worms were present. The radioactivity levels in schistosomes recovered by perfusion were then measured and here again a strong positive linear relationship was found between these levels and the number of worms (45). This work therefore showed that adult schistosomes can be successfully imaged *in vivo* using PET to assess worm burden. However, quantification of worm burdens by PET was more limited in mice with low worm burdens.

To further validate the PET imaging approach, infected mice were treated with the drug praziquantel (PZQ). As predicted, the FDG signal measured in treated animals decreased after three days of treatment while that in untreated animals increased over the same period (45).

Since PET imaging is in wide use in human medicine, it was concluded that PET analysis for schistosomiasis may be useful to assess treatment outcome in a non-invasive manner using ROI-based analysis to assess the number of worms before and after treatment. Future investigations aiming at limiting background signal and minimizing non-specific FDG uptake will help in these efforts as will the development of more specific radiotracers.

Detecting schistosomes *in vivo* using fluorescence molecular tomography (FMT)

Intravascular schistosomes can also be visualized *in vivo* using fluorescence molecular tomography (FMT) (46). FMT detects and quantifies near-infrared probes called fluorochromes. Tomographic slicing through a live animal allows fluorochrome distribution to be assessed and quantified at all tissue depths. Computer software acquires the FMT images to determine fluorochrome concentration and distribution in three-dimensional regions of interest (ROIs) within the animals. These 3D maps of fluorescence signal can be combined with anatomical computed tomography images to more finely assign signal distribution. Fluorochromes used in FMT imaging are often protease activated and the use of FMT to image schistosomes relies on the fact that the adult worms express a battery of proteases in their intestines. Indeed, one of the adaptations of schistosomes to life in the blood is their upregulation of a collection of digestive enzyme genes following invasion of the mammalian host (47). For FMT, blood-feeding schistosomes ingest fluorochrome with the blood meal and the fluorchrome is cleaved and thereby activated by the parasite's gut cathepsins. About 24 hours after i.v. injection of the fluorchrome ProSense 680, schistosome-infected mice have been subjected to quantitative tomographic analysis via FMT (46).

Quantitation of the total signal detected in the *S. mansoni* infected versus uninfected mice revealed significantly higher signal in the infected mice with the signal reflecting the localization of the parasites within the infected mice (46). Not surprisingly, the parasites were not uniformly distributed throughout the abdomen but were accumulated in selected areas. A major site of accumulation was the upper mesenteric veins with an area of minor parasite accumulation in the portal vein.

After imaging, parasites were perfused from the vasculature and counted; linear regression analysis revealed a significant and strong positive correlation between worm numbers and *in vivo* fluorescence signal intensity (46). As few as 3 worms could be detected *in vivo*. When the male and female *S. mansoni* worms recovered by perfusion were examined by near-infrared fluorescence microscopy all exhibited vivid fluorescence throughout the length of their bifurcated guts. This is consistent with the notion that the parasites did ingest and activate the fluorchrome. In a similar vein, the remaining important human schistosome parasites, *S. japonicum* and *S. hematobium*, when similarly treated, also exhibited extensive and strong gut fluorescence when recovered from infected animals (46). These data suggest that the technology will permit investigators to gain direct visual insight into the behavioral biology of the major schistosome parasites of humans including factors that influence their intravascular migration, dissemination and distribution within the host.

To assess the utility of *in vivo* imaging by FMT to test anti-schistosome therapeutics, infected animals were treated with PZQ and, one week later, imaging was performed on all mice, including an untreated, infected control group and an uninfected, control group. It was found that a substantially greater signal was detected in the untreated group reflecting the large worm burden in these animals. In contrast, the PZQ-treated group, cured of their infection, exhibited comparably low levels of fluorescence as seen in the control uninfected group (46). Therefore this technique, like PET above, may be useful in rapidly assessing the efficacy of novel anthelminthics.

Examining schistosomes *in vivo* using intravital microscopy (IVM)

With PET and FMT, images of schistosomes are generated from signals emanating from the parasites *in vivo*. To directly monitor schistosomes within their hosts, intravital microscopy

(IVM) has been used (7). IVM is a technique that employs microscopy to observe biological systems *in vivo* at high resolution. Advanced microscopy capabilities have raised the profile of this methodology but it is not new and in its more basic form has been utilized to examine *S. mansoni* worms residing inside infected experimental animals. Here, the internal organs of anesthetized, schistosome-infected mice were surgically exposed, irrigated with warm isotonic fluid, covered with a transparent sheet and examined microscopically (7). Between the fifth and seventh day after infection, “masses equivalent to the size of” schistosomula were observed passing rapidly through pulmonary arterioles and, as soon as the eight day after infection, were detected in the liver (7).

The feeding pattern of numerous parasites was directly observed in the portal vasculature. All worms faced against the direction of blood flow and fed by opening and closing the oral cavity every 1–2 seconds. Such feeding movements were either continuous for the observed period (1 hour) or lasted ~10 minutes to be repeated after a rest period of about the same time length. During feeding the worms were attached to the blood vessel wall by their ventral sucker or were observed to be wedged in the vessel. Worms tended not to move during feeding bouts. Between feeding, some worms migrated a few hundred microns along the blood vessel wall (7). As parasites fed and increased in size, some were observed by IVM to impede blood flow. This interference with blood flow was restricted to the branch of the interlobular portal venule in which they were feeding. Accompanying the reduction in blood flow in these vessels, blood flow was also reduced in the adjacent sinusoids whose walls become dilated or sacculated (7).

Single and paired worms were observed in the portal vein and in various branches of the superior and inferior mesenteric veins (7). Both single and paired worms moved constantly along these vessels, usually in an undulating fashion against the normal direction of blood flow. As worms entered veins and venules that were equivalent to their diameter, they elongated sufficiently to do so (7). While the worms could move and were feeding, it is not known whether the anesthetic administered to the mice impacted the normal behavior of the parasites.

Schistosome eggs were observed in the walls of venules of the small and large intestine. Their attachment was firm since attempts to dislodge them by repeated compression of the blood vessel seldom succeeded. As blood circulated against the eggs, no elements of the blood adhered to their surfaces and as single eggs passed through the wall of the vessel and into the parenchyma, no inflammatory response was noted. Clusters of eggs were observed to occlude blood flow which produced localized necrosis of the vessel wall with attendant hemorrhage (7).

Detecting schistosome eggs in vivo using confocal laser scanning microscopy (CLSM)

Recently, confocal laser scanning microscopy (CLSM) has been introduced as a diagnostic tool to image schistosome eggs. Exploiting a laser system designed for scanning a living eye, *S. mansoni* eggs were first detected within the mucosa of dissected mouse guts and later in the colons of infected mice. Employing a rigid endoscope permitted an examination of schistosome eggs within different areas along the length of the gut of each mouse (48). Clear visualization of the shape of the eggs *in situ* permits an identification of the species present. Additionally, miracidial twitching movements can be seen, along with movement of the flame cells and the degree of development of the miracidia which gives a strong indication of parasite viability. The technique has also been applied to successfully detect *S. hematobium* eggs in the mucosal tissue of the bladder of a human patient with schistosomiasis (49). Advantages of CLSM for diagnosis include the fact that the technique

is considered non-invasive and allows a larger tissue area to be scanned for eggs than does a biopsy.

In summary, a number of different imaging modalities have been used to examine schistosomes and monitor schistosomiasis. Intravital microscopy (IVM) allows direct visual examination of the worms within the host blood vessels, albeit in an anaesthetized animal following surgical exposure of the infected vasculature. Indirect and non-invasive imaging methods to monitor schistosomes *in vivo* include positron emission tomography (PET) and fluorescence molecular tomography (FMT). These depend on the uptake and concentration of tracers (e.g. radionuclides or fluorochromes) by the parasites which are detected externally. While less intrusive than IVM, both PET and FMT must contend with the fact that healthy tissues can also take up tracer and this can lead to high backgrounds. Finally, ultrasound is a non-invasive imaging method that has long been used not to detect schistosomes but rather to assess schistosome-induced pathology. It seems likely that further advances in imaging modalities will provide greater insight into disease progression and into the biology of schistosomes in the most relevant setting – within an infected person or an experimentally infected live animal.

Acknowledgments

This work was supported by National Institutes of Health grant AI-056273.

References

1. Gryseels B. Schistosomiasis. Infectious disease clinics of North America. 2012; 26:383–397. [PubMed: 22632645]
2. Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. Acta tropica. 2000; 77:41–51. [PubMed: 10996119]
3. McKerrow JH, Salter J. Invasion of skin by Schistosoma cercariae. Trends in parasitology. 2002; 18:193–195. [PubMed: 11983589]
4. Hockley DJ, McLaren DJ. *Schistosoma mansoni*: changes in the outer membrane of the tegument during development from cercaria to adult worm. International journal for parasitology. 1973; 3:13–25. [PubMed: 4687430]
5. Wilson RA. The saga of schistosome migration and attrition. Parasitology. 2009; 136:1581–1592. [PubMed: 19265564]
6. Ross AG, Bartley PB, Sleigh AC, et al. Schistosomiasis. The New England journal of medicine. 2002; 346:1212–1220. [PubMed: 11961151]
7. Bloch EH. In vivo microscopy of schistosomiasis. II. Migration of *Schistosoma mansoni* in the lungs, liver, and intestine. The American journal of tropical medicine and hygiene. 1980; 29:62–70. [PubMed: 7352629]
8. Keating JH, Wilson RA, Skelly PJ. No overt cellular inflammation around intravascular schistosomes in vivo. The Journal of parasitology. 2006; 92:1365–1369. [PubMed: 17304823]
9. deWalick S, Tielens AG, van Hellemond JJ. *Schistosoma mansoni*: the egg, biosynthesis of the shell and interaction with the host. Experimental parasitology. 2012; 132:7–13. [PubMed: 21840309]
10. Boros DL. Immunopathology of Schistosoma mansoni infection. Clinical microbiology reviews. 1989; 2:250–269. [PubMed: 2504481]
11. Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. Nature reviews Immunology. 2002; 2:499–511.
12. Grimaud JA, Borojevic R. Portal fibrosis: intrahepatic portal vein pathology in chronic human schistosomiasis mansoni. Journal of submicroscopic cytology. 1986; 18:783–793. [PubMed: 3097335]
13. Cheever AW. Differential regulation of granuloma size and hepatic fibrosis in schistosome infections. Memorias do Instituto Oswaldo Cruz. 1997; 92:689–692. [PubMed: 9566240]

14. Meurs L, Mbow M, Vereecken K, Menten J, Mboup S, Polman K. Bladder morbidity and hepatic fibrosis in mixed *Schistosoma haematobium* and *S. mansoni* Infections: a population-wide study in Northern Senegal. PLoS neglected tropical diseases. 2012; 6:e1829. [PubMed: 23029589]
15. Cheever AW, Kamel IA, Elwi AM, Mosimann JE, Danner R. *Schistosoma mansoni* and *S. haematobium* infections in Egypt. II. Quantitative parasitological findings at necropsy. The American journal of tropical medicine and hygiene. 1977; 26:702–716. [PubMed: 889013]
16. Cheever AW, Andrade ZA. Pathological lesions associated with *Schistosoma mansoni* infection in man. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1967; 61:626–639. [PubMed: 4293432]
17. Mohamed-Ali Q, Elwali NE, Abdelhameed AA, et al. Susceptibility to periportal (Symmers) fibrosis in human *Schistosoma mansoni* infections: evidence that intensity and duration of infection, gender, and inherited factors are critical in disease progression. The Journal of infectious diseases. 1999; 180:1298–1306. [PubMed: 10479161]
18. Warren KS. The pathogenesis of “clay-pipe stem cirrhosis” in mice with chronic schistosomiasis mansoni, with a note on the longevity of the schistosomes. The American journal of pathology. 1966; 49:477–489. [PubMed: 5920326]
19. Palmer, PES.; Reeder, MM. In The Imaging of Tropical Diseases. New York: Springer-Verlag; 1981. p. 109-204.
20. Hatz C, Murakami H, Jenkins JM. A review of the literature on the use of ultrasonography in schistosomiasis with special reference to its use in field studies. 3*Schistosoma japonicum*. Acta tropica. 1992; 51:29–36. [PubMed: 1351353]
21. Ohmae H, Sy OS, Chigusa Y, Portillo GP. Imaging diagnosis of schistosomiasis japonica--the use in Japan and application for field study in the present endemic area. Parasitology international. 2003; 52:385–393. [PubMed: 14665397]
22. Hatz C, Savioli L, Mayombana C, Dhunputh J, Kisumku UM, Tanner M. Measurement of schistosomiasis-related morbidity at community level in areas of different endemicity. Bulletin of the World Health Organization. 1990; 68:777–787. [PubMed: 2127383]
23. Strahan R, Chiyesu KO, Schneider-Kolsky ME. Ultrasound study of liver disease caused by *Schistosoma mansoni* in rural Zambian schoolchildren. Journal of medical imaging and radiation oncology. 2012; 56:390–397. [PubMed: 22883646]
24. King CH, Magak P, Salam EA, et al. Measuring morbidity in schistosomiasis mansoni: relationship between image pattern, portal vein diameter and portal branch thickness in large-scale surveys using new WHO coding guidelines for ultrasound in schistosomiasis. Tropical medicine & international health: TM & IH. 2003; 8:109–117. [PubMed: 12581434]
25. Richter, J.; Hatz, C.; Capangne, G.; Bergquist, N.; Jenkins, J. Geneva: World Health Organization. 2000.
26. Pinto-Silva RA, Queiroz LC, Azeredo LM, Silva LC, Lambertucci JR. Ultrasound in schistosomiasis mansoni. Memorias do Instituto Oswaldo Cruz. 2010; 105:479–484. [PubMed: 20721494]
27. Barata CH, Pinto-Silva RA, Lambertucci JR. Abdominal ultrasound in acute schistosomiasis mansoni. The British journal of radiology. 1999; 72:949–952. [PubMed: 10673945]
28. Doehring-Schwerdtfeger E, Mohamed-Ali G, Abdel-Rahim IM, et al. Sonomorphological abnormalities in Sudanese children with *Schistosoma mansoni* infection: a proposed staging-system for field diagnosis of periportal fibrosis. TheAmerican journal of tropical medicine and hygiene. 1989; 41:63–69.
29. Jenkins JM, Hatz C. The use of diagnostic ultrasound in schistosomiasis--attempts at standardization of methodology. Cairo Working Group. Acta tropica. 1992; 51:45–63. [PubMed: 1351355]
30. Wiest PM, Wu G, Zhang S, et al. Morbidity due to schistosomiasis japonica in the People’s Republic of China. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1992; 86:47–50. [PubMed: 1566304]
31. Schwartz DA. Helminths in the induction of cancer II. *Schistosoma haematobium* and bladder cancer. Tropical and geographical medicine. 1981; 33:1–7. [PubMed: 7018036]

32. Thomas JE, Bassett MT, Sigola LB, Taylor P. Relationship between bladder cancer incidence, *Schistosoma haematobium* infection, and geographical region in Zimbabwe. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1990; 84:551–553. [PubMed: 2128668]
33. Koukounari A, Donnelly CA, Sacko M, et al. The impact of single versus mixed schistosome species infections on liver, spleen and bladder morbidity within Malian children pre-and post-praziquantel treatment. BMC infectious diseases. 2010; 10:227. [PubMed: 20670408]
34. Koukounari A, Sacko M, Keita AD, et al. Assessment of ultrasound morbidity indicators of schistosomiasis in the context of large-scale programs illustrated with experiences from Malian children. The American journal of tropical medicine and hygiene. 2006; 75:1042–1052. [PubMed: 17172363]
35. Homeida MA, el Tom I, Nash T, Bennett JL. Association of the therapeutic activity of praziquantel with the reversal of Symmers' fibrosis induced by *Schistosomamansoni*. The American journal of tropical medicine and hygiene. 1991; 45:360–365. [PubMed: 1928571]
36. Cota GF, Pinto-Silva RA, Antunes CM, Lambertucci JR. Ultrasound and clinical investigation of hepatosplenic schistosomiasis: evaluation of splenomegaly and liver fibrosis four years after mass chemotherapy with oxamniquine. The American journal of tropical medicine and hygiene. 2006; 74:103–107. [PubMed: 16407352]
37. Ohmae H, Tanaka M, Hayashi M, et al. Improvement of ultrasonographic and serologic changes in *Schistosoma japonicum*-infected patients after treatment with praziquantel. The American journal of tropical medicine and hygiene. 1992; 46:99–104. [PubMed: 1536391]
38. EJ, Hsiang M, Zhang Y, Johnson S, Hubbard A, Spear RC. The impact of *Schistosoma japonicum* infection and treatment on ultrasound-detectable morbidity: a five-year cohort study in Southwest China. PLoS neglected tropical diseases. 2010; 4:e685. [PubMed: 20502515]
39. Cheng PC, Chiang PF, Lee KM, et al. Evaluating the potential of a new isotope-labelled glyco-ligand for estimating the remnant liver function of schistosoma-infected mice. Parasite immunology. 2013; 35:129–139. [PubMed: 23216139]
40. Bueding E. Carbohydrate metabolism of *Schistosoma mansoni*. The Journal of general physiology. 1950; 33:475–495. [PubMed: 15422103]
41. Skelly PJ, Shoemaker CB. Rapid appearance and asymmetric distribution of glucose transporter SGTP4 at the apical surface of intramammalian-stage *Schistosoma mansoni*. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93:3642–3646. [PubMed: 8622989]
42. Jiang J, Skelly PJ, Shoemaker CB, Caulfield JP. *Schistosoma mansoni*: the glucose transport protein SGTP4 is present in tegumental multilamellar bodies, discoid bodies, and the surface lipid bilayers. Experimental parasitology. 1996; 82:201–210. [PubMed: 8617347]
43. Zhong C, Skelly PJ, Leaffer D, Cohn RG, Caulfield JP, Shoemaker CB. Immunolocalization of a *Schistosoma mansoni* facilitated diffusion glucose transporter to the basal, but not the apical, membranes of the surface syncytium. Parasitology. 1995; 110 (Pt 4):383–394. [PubMed: 7753579]
44. Skelly PJ, Kim JW, Cunningham J, Shoemaker CB. Cloning, characterization, and functional expression of cDNAs encoding glucose transporter proteins from the human parasite *Schistosoma mansoni*. The Journal of biological chemistry. 1994; 269:4247–4253. [PubMed: 8307988]
45. Salem N, Balkman JD, Wang J, et al. In vivo imaging of schistosomes to assess disease burden using positron emission tomography (PET). PLoS neglected tropical diseases. 2010:4.
46. Krautz-Peterson G, Ndegwa D, Vasquez K, et al. Imaging schistosomes *in vivo*. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2009; 23:2673–2680. [PubMed: 19346298]
47. Tort J, Brindley PJ, Knox D, Wolfe KH, Dalton JP. Proteinases and associated genes ofparasitic helminths. Advances in parasitology. 1999; 43:161–266. [PubMed: 10214692]
48. Holtfreter MC, Stachs O, Reichard M, Loebermann M, Guthoff RF, Reisinger EC. Confocal laser scanning microscopy for detection of *Schistosoma mansoni* eggs in the gut of mice. PloS one. 2011; 6:e18799. [PubMed: 21533168]
49. Fritzsche C, Stachs O, Holtfreter MC, Nohr-Luczak C, Guthoff RF, Reisinger EC. Confocal laser scanning microscopy, a new in vivo diagnostic tool for schistosomiasis. PloS one. 2012; 7:e34869. [PubMed: 22529947]