Review

The chronic enteropathogenic disease schistosomiasis

David U. Olveda [a], Remigio M. Olveda [b], Donald P. McManus [c], Pengfei Cai [c], Thao N.P. Chau [d], Alfred K. Lam [a], Yuesheng Li [c], Donald A. Harn [e], Marilyn L. Vinluan [b], Allen G.P. Ross [a,*]

[a] Department of Medical Sciences, Griffith Health Institute, Gold Coast, Australia
[b] Department of Health, Research Institute for Tropical Medicine, Manila, The Philippines
[c] Department of Molecular Parasitology, QIMR Berghofer Medical Research Institute, Brisbane, Australia
[d] Department of Public Health, Flinders University, Adelaide, Australia
[e] Department of Infectious Diseases, University of Georgia, Georgia, USA

S U M M A R Y

Schistosomiasis is a chronic enteropathogenic disease caused by blood flukes of the genus Schistosoma. The disease afflicts approximately 240 million individuals globally, causing approximately 70 million disability-adjusted life years lost. Chronic infections with morbidity and mortality occur as a result of granuloma formation in the intestine, liver, or in the case of Schistosoma haematobium, the bladder. Various methods are utilized to diagnose and evaluate liver fibrosis due to schistosomiasis. Liver biopsy is still considered the gold standard, but it is invasive. Diagnostic imaging has proven to be an invaluable method in assessing hepatic morbidity in the hospital setting, but has practical limitations in the field. The potential of non-invasive biological markers, serum antibodies, cytokines, and circulating host microRNAs to diagnose hepatic fibrosis is presently undergoing evaluation. This review provides an update on the recent advances made with respect to gastrointestinal disease associated with chronic schistosomiasis.

1. Introduction

Schistosomiasis (bilharzia) is a chronic enteropathogenic disease caused by blood flukes of the genus Schistosoma.[1] It affects approximately 240 million people and is considered the third most devastating tropical disease in Africa, South America, the Caribbean, the Middle East, and Asia.[2–4] More than 78 countries are affected, and nearly 800 million people are at risk of infection.[3,4] Of the five species that infect humans, Schistosoma mansoni, Schistosoma haematobium, and Schistosoma japonicum cause the most morbidity.[3,5] The disease burden due to these three species is estimated to be as high as 29 million disability-adjusted life years (DALYs).[6]

The lifecycles of the five schistosome species are similar and involve a snail intermediate host (Figure 1). Chronic infections with all Schistosoma species, with the exception of S. haematobium, can cause significant morbidity and mortality as a result of granuloma formation in the intestine and liver.[7] However, cases of liver[8] and intestinal disease[9] from S. haematobium have also been reported. This review emphasizes the pathogenesis of the intestinal and hepatosplenic forms of the disease – the major causes of morbidity in schistosomiasis mansoni and japonica. The evaluation of hepatic fibrosis with diagnostic imaging and the performance of different direct serum biomarkers and potential use of circulating microRNAs (miRNAs) for disease staging and predicting the risk of hepatic fibrosis are also discussed.

2. Chronic schistosomiasis

2.1. Intestinal disease

One of the common manifestations of the chronic form of this enteropathogenic disease is intestinal schistosomiasis. The infection is caused by S. mansoni, S. japonicum, S. mekongi, and S.
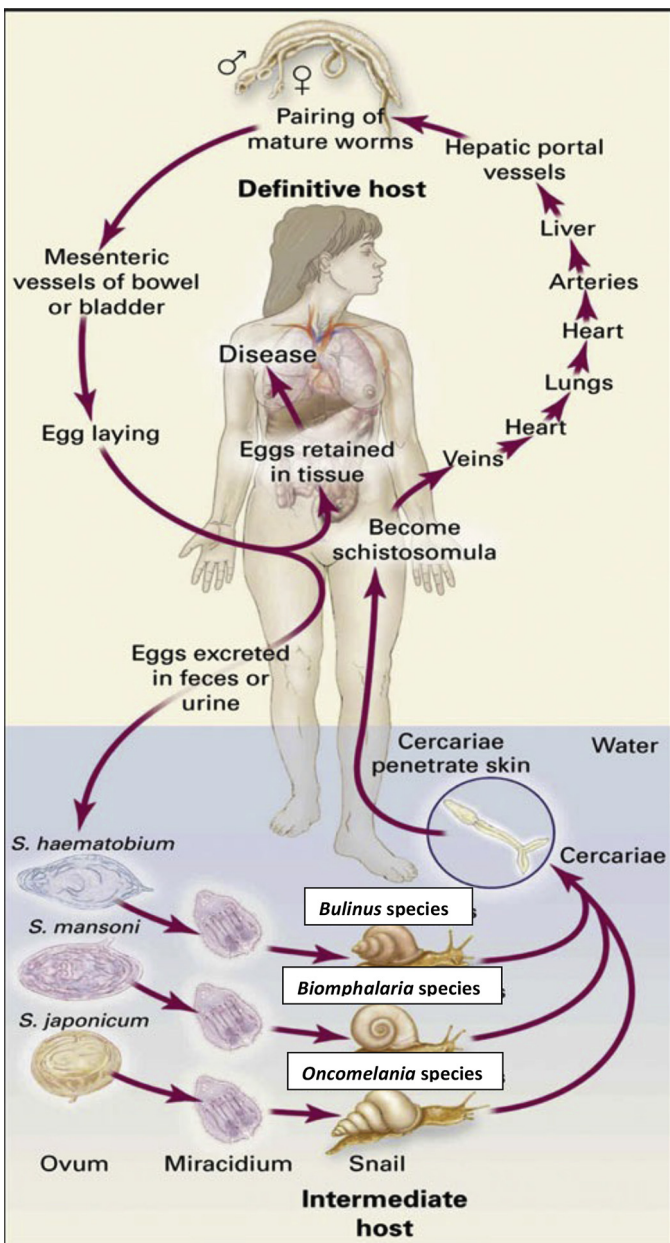
Figure 1. Schistosome lifecycle (Ross AG, McManus DP, Farrar J, Hunstman RJ, Gray DJ, Li YS, et al. Neuroschistosomiasis. J Neurol 2012; 259:22–32).

of calcified eggs, atrophy of the overlying mucosa ensues and it acquires a granular dirty yellowish appearance.[15] Polyps, which are said to be the most common among the spectrum of intestinal lesions,[16] may result from an immune-mediated inflammatory process associated with continued egg deposition[17] and ova entrapment leading to a foreign body reaction with progressive inflammation and fibrosis. Schistosomal eggs are deposited in the superficial layers of the submucosa where reactive cellular debris and vascular granulation tissue accumulate. Eggs will then produce a cell-mediated inflammatory response with granuloma formation and necrosis. The subsequent healing of the necrotic foci will lead to the formation of fibrous connective tissue and hypertrophy of the muscularis mucosa. The fibrous connective tissue in the submucosa and the hypertrophied muscularis mucosa form a barrier to the ova transiting from the mesenteric veins to the gut lumen. The trapped ova then elicit further inflammation and fibrosis. This continuous process elevates the hypertrophied muscularis mucosa to form a nodule which is the earliest detectable polyp.[18]

Clinical manifestations of intestinal schistosomiasis include abdominal pain, altered bowel habits, and bloody stools.[5] Iron-deficiency anemia and eosinophilia are also present.[19] Polyposis from intestinal schistosomiasis does not appear to be related with colorectal carcinoma,[20,21] but a recent study has shown that a history of colonic schistosomiasis japonica is a probable independent risk factor for the development of colorectal neoplasias.[22]

Appendiceal schistosomiasis was first documented in 1909, and the most frequent species associated with this condition are *S. haematobium* and *S. mansoni*. In one case report, schistosomiasis haematobia presented as acute appendicitis in a 26-year-old Israeli male who developed symptoms 2 years after visiting Africa; tissue sections showed extensive inflammatory areas and fibrosed granulomas.[9] This rare condition was also reported recently in a 30-year-old male UK resident from Ghana; histological sections of his appendix revealed luminal pus associated with numerous *S. mansoni* egg masses transmurally and within the subserosal adipose tissue. The usual granulomatous response around the eggs was evident. Eggs in the submucosa produce an obstructive type of appendicitis, while serosal lesions produce inflammation and adhesion formation.[23]

In Saudi Arabia, an unusual case of disseminated peritoneal *S. japonicum* has also been reported in a 32-year-old Filipino female who presented with signs and symptoms of acute appendicitis. However, a right iliac fossa mass was also seen on diagnostic laparoscopy. Microscopic sections of both the appendiceal wall and the adherent omental mass showed suppurative inflammation and multiple foci of schistosomal ova highly indicative of the *S. japonicum* species. Interestingly, a granulomatous response was not seen in the sections examined.[24]

intercalatum.[10] Some cases of *S. haematobium* and *S. guineensis* infection have also been reported.[11,12] The pathology associated with intestinal schistosomiasis is due to egg deposition and granuloma formation, which eventually leads to acute then chronic schistosomal colitis and polyp formation.[13] Although areas in both the small and large intestine may be involved, most severe lesions are found in the large intestine. It is theorized that the adult worms have a tendency to inhabit the branches of the inferior mesenteric vein and superior hemorrhoidal vein; hence, more eggs are deposited in the large intestine, especially in the rectum, sigmoid, and descending colon.[14]

Ova are generally distributed in the loose submucosa of the large intestine and to a lesser extent in the subserosa. The muscularis mucosa subsequently becomes involved, and the underlying mucosa may either undergo hyperplastic changes or be denuded and form small superficial ulcers. When the submucosa becomes heavily thickened with fibrous tissue containing massive amounts

2.2. Hepatosplenic disease

Hepatic schistosomiasis represents the best known form of chronic disease with a wide range of clinical manifestations, and its pathogenesis is related to the host cellular immune response.[13] The mechanisms involved in granuloma formation and fibrosis have been documented extensively in experimental models and humans infected with *S. mansoni* and *S. japonicum*. Eggs trapped in the pre-sinusoidal portal venules secrete soluble egg antigens which are taken up by antigen-presenting cells such as macrophages.[25] Subsequently, antigen presentation stimulates Th1 cells (CD4+ T lymphocytes) to secrete interleukin (IL)-2, interferon gamma (IFN-γ), and tumor necrosis factors (TNF), which in turn drive a cell-mediated response and attract more immune cells around the ova. As the granuloma becomes more organized, the Th1 cells are gradually replaced by Th2 cells, which produce IL-4, IL-5, IL-10, and IL-13, completing granuloma maturation.[26]

Towards the late stage of granuloma formation, the fibroblasts are stimulated by egg products and by T lymphocyte cytokines to proliferate, replacing most of the cellular elements, and mediating fibrotic collagenous material deposition around the portal vein tributaries. The pathogenesis of hepatic fibrosis leading to hepatosplenic schistosomiasis is illustrated for *S. japonicum* in Figure 2.

Fibrosis, leading to portal hypertension, is the major cause of disease morbidity and mortality. Grossly, whitish plaques known as 'clay-pipestem' fibrosis are evident on cut sections, contrasting with the intact liver acinar architecture.[27] Lesions commence as eosinophilic infiltrates surrounding trapped eggs, which may subsequently lead to abscess formation. A Splendore–Hoeppli reaction (asteroid body formation) may sometimes occur.[28] Peri-ovular granulomas develop and the eggs inside degenerate and calcify over time. As older granulomas involute, macrophage-predominant granulomas begin to form.[27] Eventually, granulomas are replaced by surrounding fibrous tissue. As more new eggs arrive, resultant damage to larger diameter veins occurs, along with periportal granulomatous inflammation and inter-granulo-matous fibrosis. In the absence of a coexisting hepatotropic viral infection, the liver of patients with periportal fibrosis secondary to schistosomiasis retains its hepatocellular function, differentiating the disease from cirrhosis and other liver diseases.[7,29] Severe schistosome-induced hepatic fibrosis causes portal vein obliteration leading to the development of portal hypertension, and lethal complications of the hepatosplenic form of the disease include pulmonary hypertension,[30,31] glomerulopathy,[32,33] splenomegaly, and thrombocytopenia.[34]

Despite the general mechanism of granuloma formation leading to hepatic fibrosis in schistosomiasis, studies have shown some peculiarities regarding this pathogenesis among schistosome species. For instance, in a study involving mouse models, tissue studies were done to shed light on the characteristics of granulomas caused by *S. mekongi* compared with *S. japonicum*-induced granulomas. In the murine livers, it was shown that *S. mekongi*-induced granulomas were initially cellular, formed by foam cells, and continuously appeared in the intralobular areas, while *S. japonicum*-induced granulomas were fibrous and did not continuously appeared in the intralobular areas. Portal fibrosis was also not observed in *S. mekongi*-infected murine livers, while this lesion appeared later in livers infected with *S. japonicum*. It was thought that the absence of portal fibrosis in *S. mekongi* infection allowed the eggs to infiltrate the interlobular areas continuously, which may have accounted for the absence of echogenic pattern on ultrasonographic evaluation (a feature noted with *S. japonicum* species).[35] On the other hand, in another study, the intensity of liver fibrosis among patients with *S. mekongi* infection was assessed by liver biopsy. Liver biopsies revealed complete disorganization of hepatic architecture with fibrous enlargement of portal tracts and some portal–portal bridging fibrosis, but there was no cirrhosis. There was blood vessel congestion and thrombosis with inflammation in the portal areas. Numerous eggs of *S. mekongi* were observed mostly in fibrous areas and more rarely in the parenchyma. Some eggs were surrounded by epithelioid and giant cell reaction. A high degree of fibrosis was observed among young adults in that study and this contrasts with the findings observed with other schistosome species.[36]
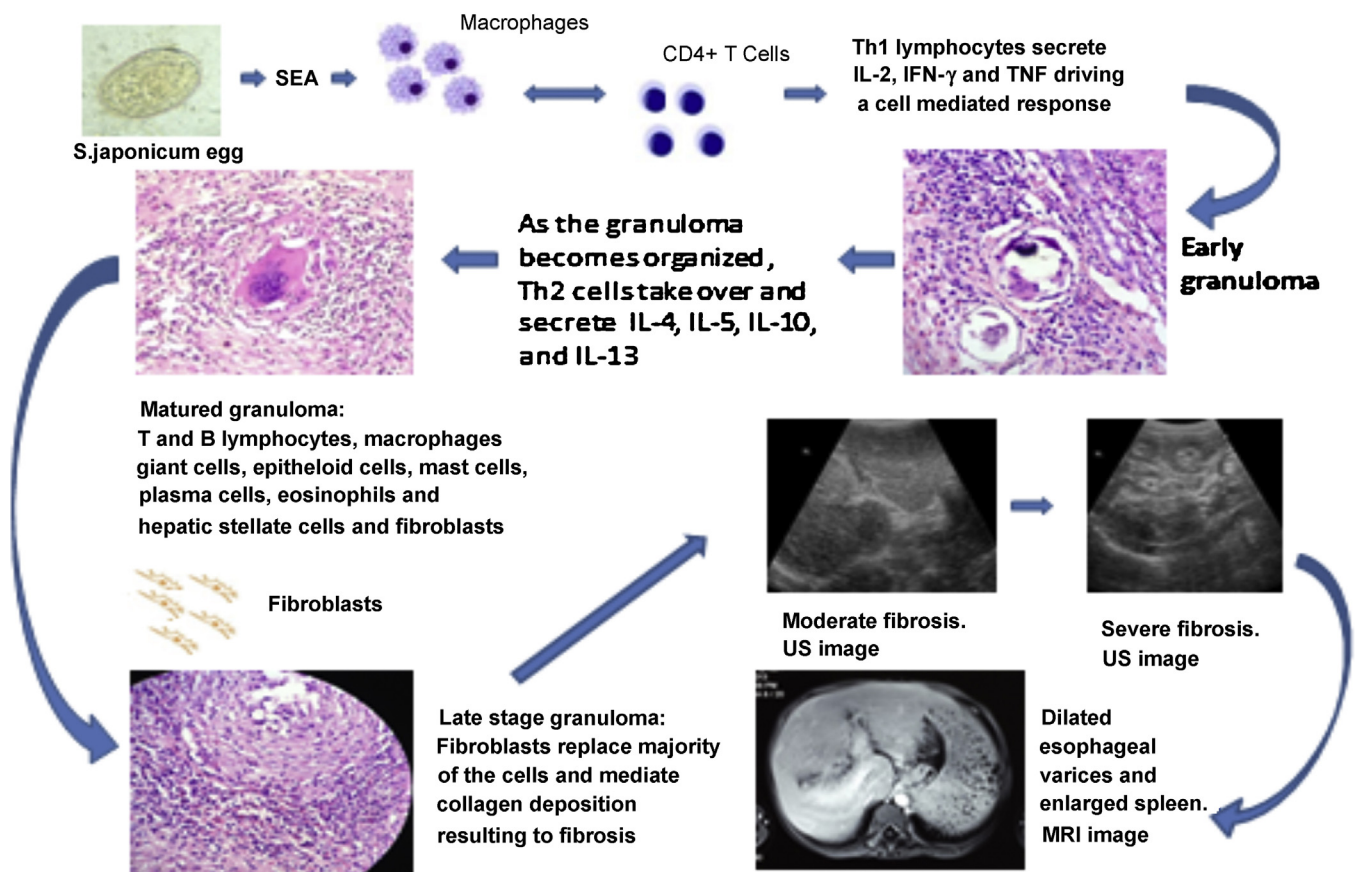

Figure 2. Pathogenesis of hepatic fibrosis leading to hepatosplenic schistosomiasis.

The clinical manifestations of S. mekongi are similar to those of S. mansoni and S. japonicum infections.[37] Clinical hepatomegaly, splenomegaly, reported blood in stool,[38,39] abdominal pain,[38] diverted circulation, and ascites[39] are the frequent reported clinical signs in studies assessing hepatosplenic morbidity from S. mekongi.

Although several studies have clearly shown that schistosome egg-induced granulomas are pathogenic to the host, there are some current studies suggesting that the lesions may have some protective functions. In an S. mansoni study[40] involving transgenic mice, it was thought that granuloma formation around the egg offered some protection against schistosome-related hepatotoxicity (an occurrence that is currently observed only in S. mansoni-infected mice). Mice that were not able to produce the necessary cytokines to form a functional granuloma died early due to egg-induced endotoxemia. The granuloma is not only thought to act as a physical barrier, but also functions to sequester the antigenic products secreted by the egg. Nevertheless, it is also thought that the parasite takes advantage of this functional granuloma by facilitating excretion of its eggs, without killing the host, and therefore continuing its lifecycle.[41]

Recently, hepatic stellate cells (HSCs) have been acknowledged to be major players in the liver fibrotic process.[42] The interactions of the HSCs with S. japonicum[43] and S. mansoni eggs[44] have been investigated; both studies supported the contributory role of these cells in schistosome-induced hepatic fibrosis. Further, it has been demonstrated that the activated HSC myofibroblastic phenotype can be reversed to its quiescent phenotype (manifested by lipoprotein storage in cells) by S. mansoni egg antigens, an interaction not observed with S. japonicum eggs. This inability of the S. japonicum eggs to induce a quiescent myofibroblastic phenotype has been suggested to explain in part why S. japonicum is more pathogenic to the liver compared with S. mansoni.[45]

3. Evaluation of hepatic fibrosis

The pathology of schistosome-induced liver disease has been studied extensively since its first description in 1904.[46] The term 'Symmers fibrosis' was originally adopted to describe the unusual pattern of collagen and glycosaminoglycan (GAG) deposition observed in an autopsy. This term was changed in 1947 to clay-pipestem fibrosis in recognition of the fact that the hepatic parenchyma was spared in schistosomal-induced liver disease and appeared fundamentally different from 'cirrhotic' deposits of collagen and GAGs.[47]

Currently there are a number of methods that can be used to diagnose and evaluate the severity of liver fibrosis. Liver biopsy is considered the gold standard but causes significant discomfort and possible post-procedural risk. The evaluation of tissue morphology may be informative but does not have sufficient sensitivity to diagnose periportal fibrosis (PPF) and cannot explain the disease dynamics occurring between sampling periods.[48] Imaging modalities like ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI), on the other hand, are used not only to support the diagnosis of schistosomiasis but also to accurately assess and detect target organ damage that can develop due to chronic infection with schistosomiasis. Research to identify non-invasive markers for hepatic fibrosis is also underway and the combination of these biomarkers along with comprehensive history and physical examination, basic laboratory tests, and imaging methods seems to offer the best approach for evaluating patients with this disease.[49]

3.1. Diagnostic imaging

The reliability of US has made it the routine imaging method in the evaluation of hepatosplenic schistosomiasis for the past 30 years.[50–53] In the diagnosis of S. mansoni, US can demonstrate PPF appearing as echogenic tubular shadows with an anechoic lumen that radiates from the porta hepatis. When the tubular structure is viewed crosswise, it appears as a ring of concentric fibrosis surrounding portal venous vasculature, and is known as the 'bull's eye lesion'.[54–56] Other US findings include hypertrophy of the left hepatic lobe, atrophy of the right hepatic lobe, gallbladder wall thickening, granulomas, and splenic nodules. The above lesions can also be seen in liver pathology associated with S. japonicum infection. However, the demonstration of a septal formation by high echogenic bands like a mosaic or network pattern in the liver by US is typical only for S. japonicum infection.[57,58]

CT, on the other hand, is not routinely used in the evaluation of schistosomiasis due to its cost and the utilization of ionizing radiation. CT scans show similar imaging findings to US, including atrophy of the right hepatic lobe, hypertrophy of the left hepatic lobe, splenomegaly, and ascites. PPF is seen in CT as a band of low attenuation around portal vein branches throughout the liver, with enhancement following the intravenous administration of contrast medium. The 'bull's eye' lesion in the liver can be demonstrated on CT as concentric layers of periportal enhancement and is thought by some authors to be a more specific indicator of schistosomiasis than the periportal enhancement.[59] The network pattern seen in the liver in S. japonica appears on CT as a 'turtleback' or 'tortoiseshell' lesion. This lesion is thought to be due to septal calcification of schistosomal ova.[60]

In the diagnosis of hepatosplenic schistosomiasis using MRI, the most frequent findings are accentuation of periportal signal in T2-weighted sequences, and a hypointense signal in relation to the normal liver parenchyma in T1-weighted sequences with fat suppression. The periportal signal is accentuated on T1-weighted sequences following contrast administration. It has been suggested that periportal inflammation may be differentiated from fibrosis by the hyperintense signal observed in T2-weighted sequences.[61] Portal vein thrombosis (PVT), which may be due to hepatosplenic schistosomiasis, is best diagnosed by MRI.[62] Cavernous portal vein transformation due to PVT is described on MRI as small enhancing flow voids around the right portal vein in a T1-weighted image. Although MRI uses a gadolinium-based contrast, its higher cost precludes routine use.

The utility of imaging techniques in the diagnosis of schistosomiasis is demonstrated in Figure 3. A 12-year-old Filipino boy from a known schistosomiasis endemic area in the central Philippines was diagnosed by portable gray-scale US to have moderate PPF with severe splenomegaly due to schistosomiasis japonica. Further examination with MRI confirmed the PPF and showed an additional finding of PVT and cavernous transformation of the right branch of the main portal vein. Massive splenomegaly was also demonstrated on MRI. The patient was successfully treated by splenectomy.

3.2. Serum biological markers

The supporting framework of the normal and fibrotic liver comprises a group of macromolecules called extracellular matrix (ECM). In advanced stages of fibrosis, the liver contains approximately six times more ECM than normal, including collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycan.[63] Qualitative and quantitative ECM changes in liver fibrosis can be measured in the blood or urine using indirect and direct biomarkers. Direct biomarkers are classified into three groups: (1) those that measure matrix deposition: procollagen I
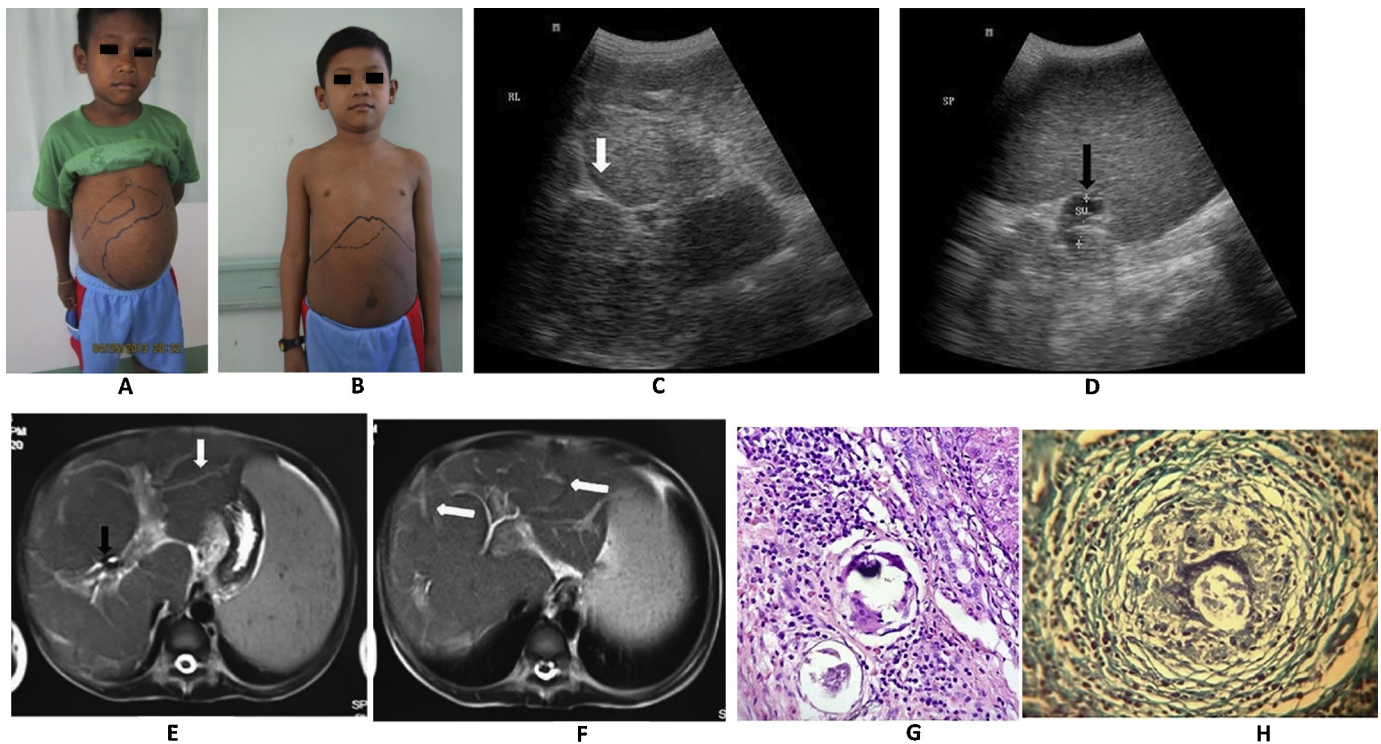
Figure 3. The abdomen of a 12-year-boy with severe schistosomiasis (A) before and (B) after splenectomy. Ultrasound of the patient showing (C) a markedly thickened branch of the main portal vein (white arrow), and (D) a markedly enlarged spleen with dilated splenic vein (black arrow). MRI depicting (E) a cavernous transformation of the right portal vein (black arrow) and periportal fibrosis running along the second branch of the portal vein (white arrow), and (F) curvilinear tracts scattered throughout the liver parenchyma consistent with periportal fibrosis (arrows). Histopathology sections showing (G) early *Schistosoma japonicum* egg granuloma and (H) late granuloma in the liver.

carboxy terminal peptide (PICP), procollagen III amino terminal peptide (PIIINP), tissue inhibitors of metalloproteinase (TIMPs), transforming growth factor beta (TGF-β), tenascin; (2) those that reflect matrix removal or degradation: procollagen IV C peptide, procollagen IV N peptide (7-S collagen), collagen IV, metalloproteinase MMP, undulin, urinary desmosine, and hydroxylysylpyridinoline; and (3) those that cannot clearly determine the relationship to the matrix deposition or removal: hyaluronic acid, YKL-40 (Chondrex), and laminin.[63,64] Many of these biomarkers have been evaluated and found useful in the diagnosis and grading of liver fibrosis caused by several conditions, including chronic viral hepatitis, alcoholic cirrhosis, non-alcoholic steatohepatitis, and schistosomiasis.[65] However, their ability to identify and grade liver fibrosis in schistosomiasis at the community level in endemic areas needs further evaluation. The performance of some direct serum markers in the evaluation of schistosome-induced hepatic fibrosis is discussed below and is summarized in Table 1.

3.3. Collagen

Collagen and its metabolic products have been examined in the blood and urine of patients with different etiologies of fibrotic liver disease.[66] Collagen types I, III, and IV have been used extensively in the evaluation of hepatic fibrosis. Serum PIIINP has also gained wide acceptance as a blood test for collagen metabolism.[67] Type III collagen is secreted in the extracellular space as a procollagen molecule. The amino terminal portion of the molecule is then cleared by enzymatic cleavage by two specific endopeptidases.[68] This releases the helical type III collagen molecule to combine with other ECM macromolecules to form a collagen fibril. This short peptide fragment (PIIIP) diffuses from fibrotic loci, circulates in the blood, and is metabolized further to form a fragment termed Col-1 (procollagen type I peptide (PIP)).[69] The PIIIP assay appears to have

its greatest utility in the early stages of fibrosis when type III collagen biosynthesis predominates.[70] In the late stages of most cirrhotic conditions, PIP is the dominant peptide synthesized.[71] A study on PICP in schistosomiasis mansoni showed that the levels of this biomarker were higher in infected patients than in uninfected controls and that serum levels decreased during the first year post-treatment with the anti-schistosome drug praziquantel; however, no correlation was established between PIP and fibrosis scores by US examination.[72] On the other hand, PIIINP levels in schistosomiasis mansoni were elevated in patients with hepatic disease compared with normal or uncomplicated control subjects. In another study it was shown that PIIINP levels returned to normal levels 18 months after patients received praziquantel.[71] Higher levels of PIIINP were also observed in subjects with advanced PPF diagnosed by histology or with more severe hepatic disease.[73]

Serum type IV collagen has also been examined in schistosomiasis patients. Levels of procollagen IV peptide did not correlate with the presence or intensity of infection, but they correlated significantly with liver fibrosis, splenomegaly, portal vein dilatation, and the presence of portosystemic collaterals.[74,75] In another study, a positive correlation between type IV collagen and advanced schistosomiasis was noted, with a significant reduction being observed in serum levels following splenectomy, but there was no correlation with the grade of PPF as determined by US.[76,77]

3.4. Hyaluronic acid

Hyaluronic acid (HA), a high molecular weight GAG, is an essential ECM component. It is synthesized by HSCs and is degraded by the sinusoidal endothelial cells.[78,79] Increased levels are due either to decreased hepatic removal or to increased production by stellate cells, or to both processes.[80] Several studies have examined HA serum levels in a number of chronic liver

Table 1
Performance of serum markers in the evaluation of hepatic fibrosis due to schistosomiasis

Serum marker	Species	Studies undertaken	Results	Reference
HA	S. mansoni	Correlation of levels of HA in patients with intense, moderate, light, and without fibrosis by US in 79 subjects	HA was able to separate individuals with fibrosis from those without, and light from intense fibrosis. The HA diagnostic accuracy for fibrosis was 0.89. With a cut-off level of 115.4 ng/ml, sensitivity and specificity were 0.98 and 0.64, respectively	Marinho et al., 2010 (Brazil)[64]
		A study of 61 patients with schistosomiasis mansoni and 16 healthy individuals	A serum HA level of 20.2 μg/l was observed that differentiated between patients with milder PPF (patterns C + D) and those with more severe PPF by US, with a sensitivity of 60% and a specificity of 65%	Silva et al., 2011 (Brazil)[87]
		Correlation of HA level in hepatosplenic schistosomiasis and with mild to advanced fibrosis	Higher levels of HA were noted in more advanced forms of schistosomiasis	Ricard-Blum et al., 1999 (Madagascar)[72]
		Serum levels in advanced forms of the disease	Serum HA levels were increased	Pascal et al., 2000[85], and Eboumbou et al., 2005 (Sudan)[126]
		Levels of HA in 153 patients in Senegal with schistosomiasis hepatic fibrosis	No changes in patient levels of HA. However 60% of patients showed early stages of hepatic involvement with US, while only 3% presented with HS disease	Burchard et al., 1998 (Senegal)[100]
	S. japonicum	HA levels in 38 cases with portal hypertension	Levels of HA are higher in those with portal hypertension than the normal range	Guangjin et al., 2002 (China)[127]
		HA levels in 193 individuals exposed to endemic Schistosoma japonicum	HA levels correlated with US findings	Li, Sleigh et al., 2000 (China)[128]
		Patients were identified with either mild (n = 30) or severe (n = 30) hepatic fibrosis due to S. japonicum infection	HA levels in normal, mild, and severe cases of hepatic fibrosis were 83.0 ± 35.7, 216.1 ± 77.9, and 212.6 ± 80.9 μg/ml, respectively. HA levels did correlate well with the degree of fibrosis.	Zheng et al., 2005 (China)[92]
TIMPs	S. japonicum	611 S. japonicum-infected Filipinos were treated with praziquantel; FibroPlex analytes produced by peripheral blood mononuclear cells stimulated with schistosome egg antigen 4 weeks after praziquantel treatment were measured and these levels related to the risk of fibrosis 1 year after praziquantel treatment	Individuals with detectable TIMP-1 had a 3.5-fold greater risk of fibrosis 1 year after praziquantel treatment compared to individuals with undetectable levels (OR 3.48; 95% CI 1.41–8.43; P = 0.007)	Fabre et al., 2011 (Philippines)[99]
Laminin	S. mansoni	Correlation of levels with portal hypertension	Laminin levels correlated with portal hypertension	Ricard-Blum et al., 1999 (Madagascar)[72]
		Field studies evaluating the levels of laminin in individuals with milder forms of schistosomiasis	Results did not reveal a correlation between laminin levels and milder forms of PPF	Kardorff et al., 1997, 1999 (Tanzania)[74,75]
		Correlation of serum laminin levels with infection (egg-positive infected patients)	Serum laminin level was significantly higher in egg-positive infected patients than in endemic controls	Tanabe et al., 1989 (Brazil)[101]; Ricard-Blum et al., 1999 (Madagascar)[72]
		Serum levels of laminin in hepatosplenic and hepatointestinal patients	Serum levels of laminin were higher in hepatosplenic than in hepatointestinal patients and were also higher in hepatointestinal patients than the controls	Parise and Rosa, 1992 (Brazil)[76]
		Laminin levels were determined in cases with initial stages of disease in hepatointestinal cases and in advanced disease	Progressive increase of laminin levels in those with initial to advanced cases of hepatointestinal schistosomiasis were observed	Wyszomirska et al., 2005 (Brazil)[76]
	S. japonicum	Laminin levels in 193 individuals exposed to endemic S. japonicum	Levels of laminin are correlated with re-infection	Sleigh et al., 2000 (China)[128]
Collagen IV	S. mansoni	Correlation of procollagen IV peptide levels and intensity of schistosomiasis, liver fibrosis, splenomegaly, portal vein dilatation, and the presence of portosystemic collaterals	Levels of procollagen IV peptide did not correlate with the presence or intensity of schistosomiasis infection, but were significantly correlated with liver fibrosis and signs of portal hypertension. Type IV collagen had a good specificity (over 90%) but poor sensitivity because more than half of those with severe liver involvement exhibited normal levels of type IV collagen	Kardorff et al., 1997, 1999 (Tanzania)[74,75]
		Correlation between type IV collagen and advanced forms of schistosomiasis mansoni	A positive correlation between type IV collagen and advanced forms of schistosomiasis mansoni. However, no correlation to the grade of PPF assessed by US examination as the gold standard method	Wyszomirska et al., 2005, 2006 (Brazil)[76]
	S. japonicum	Collagen IV levels in 193 individuals exposed to endemic S. japonicum	Levels of collagen IV correlated with re-infection	Sleigh et al., 2000 (China)[128]

Table 1 (Continued)

Serum marker	Species	Studies undertaken	Results	Reference
Procollagen type III	S. mansoni	Levels of PIIIP in 82 individuals infected with S. mansoni without liver enlargement and 20 with hepatic or hepatosplenic disease	PIIINP was elevated in patients with hepatic disease, but normal in uncomplicated cases	Zwingenberger et al., 1988 (Zaire)[71]
		Correlation of levels of PIIIP with the presence or intensity of infection or fibrosis scores	No significant correlation evident between PIIIP levels and the presence or intensity of infection or fibrosis scores	Kardorff et al., 1997, 1999 (Tanzania)[74,75]; Tanabe et al., 1989 (Brazil)[101]; Burchard et al., 1998 (Senegal)[100]; Ricard-Blum et al., 1999 (Madagascar)[72]
	S. japonicum	PIIIP levels in 38 cases with portal hypertension.	Levels of PIIIP were higher in those with portal hypertension than the normal range	Guangjin et al., 2002 (China)[127]
YKL-40	S. japonicum	Levels of YKL-40 were determined in 60 patients with mild, moderate, or severe hepatic fibrosis due to S. japonicum infection	Levels of YKL-40 were 49.0 ± 10.4, 92.3 ± 18.5, and 172.1 ± 35.9 μg/ml, respectively for mild, moderate, and severe hepatic fibrosis. Serum levels of YKL-40 correlated with the stage of hepatic fibrosis	Zheng et al., 2005 (China)[92]

HA, hyaluronic acid; US, ultrasound; PPF, periportal fibrosis; HS, hepatosplenic; TIMP, tissue inhibitor of metalloproteinase; OR, odds ratio; CI, confidence interval; PIIIP, aminoterminal procollagen III-peptide; PIIINP, procollagen III amino terminal peptide.

diseases and have suggested that it may be a candidate marker for detecting fibrosis in cirrhosis, chronic hepatitis B and C, and alcoholic and non-alcoholic fatty liver disease.[79,81–84] Studies in patients with different degrees of fibrosis due to schistosomiasis mansoni have suggested a correlation between HA serum levels and disease severity. More severe cases of PPF had much higher levels of HA, while subjects with mild fibrosis had low HA levels.[85–87] The evaluation of serum levels of HA and type IV collagen with respect to US patterns of PPF showed that only the former was capable of separating patients with mild fibrosis from those with intense fibrosis. Moreover, HA correlated positively with portal hypertension and PPF, and collateral circulation predicted HA increase.[64] It is also noteworthy that, as with serum type I collagen, the high HA levels in patients with hepatic fibrosis were reduced following praziquantel treatment.[72] In another study involving advanced schistosomiasis japonica patients and controls, serum HA and TIMP-1 levels were elevated in the former, with HA outperforming TIMP-1.[48]

3.5. Chitinase-3-like protein 1

Chitinase-3-like protein 1 (YKL-40) is a novel liver fibrosis marker. This recently described glycoprotein belongs to the chitinase family and is expressed in human liver and arthritic articular cartilage.[89–91] Although its precise physiological function is not known, YKL-40 is thought to contribute to tissue remodeling or degradation of the ECM in liver fibrosis. In a study of patients with hepatitis C virus (HCV)-associated liver disease comparing type IV collagen, PIIINP, HA, YKL-40, and biochemical parameters in the assessment of hepatic fibrosis, it was concluded that HA and YKL-40 were more useful than the other markers for assessing the fibrosis stage; in particular, YKL-40 was the most useful for monitoring the fibrosis of liver disease and for distinguishing extensive from the mild stage of liver fibrosis assessing the fibrosis stage.[89] Additionally, YKL-40 was more sensitive than HA in measuring the degree of hepatic fibrosis due to schistosomiasis; the serum levels increased in patients infected with S. japonicum and correlated with the hepatic fibrosis stage.[92]

3.6. Matrix metalloproteinases and inhibitors

The matrix metalloproteinases (MMPs) and their inhibitors are involved in controlling matrix degradation. MMPs are enzymes that are produced intracellularly and secreted as proenzymes, which require cleavage by cell surface mechanisms for functional activity. The action of MMPs is counteracted by TIMPs.[93] As such, they act to degrade ECM and to permit new matrix deposition. It has been suggested that the imbalance between MMPs and TIMPs affects the rate of fibrosis progression and that their levels correlate with the fibrosis stage, but the results have been variable and dependent upon the MMP/TIMP being assessed.[94–96]

The diagnostic accuracy of TIMP-1 and pro-MMP-2 (the free precursor molecule of MMP-2) and their relationships to histological inflammatory scores have been evaluated in patients with HCV infection; both assays performed either as well as or better than HA for the diagnosis of cirrhosis, but only TIMP-1 showed diagnostic value for the identification of patients with early stages of fibrosis. The levels of MMP-2 exhibited no relationship with the stage of fibrosis, thereby limiting its value for staging liver disease.[95,97,98] Serum MMP-1 and MMP-3 levels have also been shown not to have any diagnostic value.[94,96] On the other hand, one study has demonstrated that TIMP-1 can predict the risk of hepatic fibrosis in patients after 1 year of praziquantel treatment. This highlights the importance of identifying biomarkers for fibrosis risk because fibrosis can occur despite effective treatment.[99]

3.7. Laminin

Laminin is a major non-collagenous glycoprotein of basement membranes. Synthesized by HSCs, serum levels of laminin correlate with portal hypertension.[72] Field studies evaluating individuals with milder forms of schistosomiasis did not, however, reveal a correlation between laminin and PPF or the presence or intensity of infection.[72,74,75,100] However, some studies noted that the mean value of serum laminin was significantly higher in egg-positive schistosome-infected patients than in endemic controls.[72,101] In another study, serum laminin levels were shown to be higher in hepatic schistosomiasis subjects than in hepatointestinal patients and were also higher in the latter compared with controls.[102] A progressive increase in laminin has also been reported in the initial stages of hepatointestinal schistosomiasis and in advanced cases.[76,103]

3.8. Cytokines

Cytokines have been evaluated as biomarkers of hepatic fibrogenesis in a limited number of studies on schistosomiasis, with conflicting results. TGF-β is the dominant stimulus for producing ECM by HSCs. In a study of 88 patients who had chronic

hepatitis C, there was a correlation between total TGF-β1 and the degree of hepatic fibrosis.[103] The role of cytokines in the development of hepatic fibrosis before the hepatosplenic and early hepatosplenic stages of schistosomiasis mansoni was evaluated in a group of patients with different degrees of hepatic fibrosis determined by US. Peripheral blood mononuclear cells (PBMCs) from schistosomiasis japonica patients were stimulated by *S. japonicum* antigens and the levels of IL-5, IL-10, IL-13, IFN-γ, TNF-α, and TGF-β determined in the PBMC supernatants. Of significance, higher levels of IL-5, IL-10, and IL-13 were found in the supernatants of soluble egg antigen-stimulated PBMCs from subjects with stage III hepatic fibrosis compared to patients with stage I or II fibrosis. Significant increases in IL-5 and IL-13 levels were also observed in some of the subjects who remained untreated for 1 year following initial assessment and who developed more serious fibrosis during this period.[104] In a study of hepatosplenic schistosomiasis mansoni, there was no significant difference in the mean serum concentrations of IL-10 and IL-13 between the different categories of hepatosplenic disease.[105]

3.9. Circulating miRNAs

miRNAs comprise a family of conserved small non-coding RNAs (approximately 22 nucleotides) that can be detected in a wide range of body fluids, including blood plasma/serum. The high level of stability of miRNAs in biofluids has been attributed to two mechanisms: (1) formation of a protein–miRNA complex with argonaute proteins (mainly Ago2)[106] or high-density lipoproteins,[107] and (2) incorporation into exosomes,[108] macrovesicles, or apoptotic bodies.[109] miRNAs are being developed as novel biomarkers for various cancers and other diseases.[110–112]

For example, serum levels of liver-specific miR-122 and miR-34a were suggested to be correlated with fibrosis, steatosis, and inflammatory activities in a study of chronic HCV infection and non-alcoholic fatty liver disease (NAFLD).[113] Liver fibrosis and/or cirrhosis were also shown to be associated with increased serum levels of miR-571 and miR-513-3p and reduced serum levels of miR-29 and miR-652.[114] A recent study in HCV-infected patients has suggested that serum miR-20a may serve as a predictive biomarker for HCV-mediated fibrosis.[115] It is noteworthy that the serum exosomal miRNA expression profile was linked to grade and stages of liver fibrosis in patients with chronic HCV infection. The expression levels of two miRNAs (miR-483-5p and miR-671-5p) increased significantly and the expression levels of 14 miRNAs (let-7a, miR-106b, miR-1274a, miR-130b, miR-140-3p, miR-151-3p, miR-181a, miR-19b, miR-21, miR-24, miR-375, miR-548l, miR-93, and miR-941) were progressively reduced as liver fibrosis increased.[116]

As schistosomal egg-induced immunopathology is an unusual type of chronic liver disease distinguishable from many other liver disease types, it is tempting to speculate that a unique set of circulating miRNAs may be defined to potentially serve as sensitive molecular signatures for assessment of the severity of the fibrotic pathology of schistosomiasis. To date, a set of miRNAs of parasite origin have been identified in both *S. japonicum*[117,118] and *S. mansoni*[119,120] and miRNA expression profiles at different developmental stages of *S. japonicum* have been characterized.[121,122] However, the abundance of schistosome-specific miRNAs in the plasma of the definitive mammalian host has been shown to be relatively low, which will likely limit its utility for the diagnosis and staging of schistosomiasis.[123] Encouragingly, however, alterations of specific host miRNAs within different tissues have been shown to be associated with *S. japonicum* infection. The expression of several host miRNAs was shown to be altered rapidly in the lungs, liver, and spleen of BALB/c mice as early as 10 days post infection with *S. japonicum*.[124] A broad array of miRNAs in

liver was dysregulated and associated with the progression of *S. japonicum* infection.[125] These results raise the possibility that circulating host miRNAs may be dynamically altered during the course of schistosome infection; this provides an avenue for their development as useful diagnostic and pathogenic biomarkers for schistosomiasis, because of the strong correlation between the expression profile of miRNAs and the status/progression of the disease.

4. Conclusions

Schistosomiasis can causes significant pathology and chronic morbidity in humans infected with numerous egg granulomas and this typically results in fibrosis in target organs. The intestinal form of the disease is caused by the deposition of eggs within the bowel wall, while the hepatosplenic form of the disease is due to the eggs trapped in the liver pre-sinusoids. In the former, the eggs cause bowel lesions ranging from colitis to polyp formation, whereas in the latter, granuloma formation and subsequent fibrosis involve immune responses initially mediated by Th1 and later by Th2 lymphocytes. Severe fibrosis leads to portal hypertension-related complications causing significant illness or death.

There are various methods to diagnose and evaluate schistosome-induced liver fibrosis. Liver biopsy is still considered the gold standard, but the procedure is clinically impractical in the field. Ultrasonography is invaluable in assessing pathology, but is not readily available in many endemic communities, and the results among users can vary widely. Although costly and limited to the hospital setting, CT and MRI show distinct imaging features associated with hepatosplenic schistosomiasis and aid in the diagnosis and clinical management of patients.

Attention has recently been given to non-invasive biological markers that can determine the severity of hepatic fibrosis and monitor qualitative and quantitative changes following treatment. The most promising serum markers for the evaluation of schistosome-induced hepatic fibrosis appear to be hyaluronic acid, collagen type III, YKL-40, and laminin. More studies are needed to evaluate the utility of matrix metalloproteinases and inhibitors, and cytokines. Circulating host miRNAs show promise as useful biomarkers for schistosomiasis diagnosis due to the strong correlation between the expression profile of miRNAs and the disease status/progression. Biological markers are presently limited to research investigations and may prove too costly for broader clinical application, but their potential role in the diagnosis of schistosomiasis warrants further investigation.

References

1. Ross AG, Olds GR, Cripps AW, Farrar JJ, McManus DP. Enteropathogens and chronic illness in returning travelers. *N Engl J Med* 2013;**368**:1817–25.
2. Bockarie MJ, Kelly-Hope LA, Rebollo M, Molyneux DH. Preventive chemotherapy as a strategy for elimination of neglected tropical parasitic diseases: endgame challenges. *Philos Trans R Soc Lond B Biol Sci* 2013;**368**:2012–144.
3. Gray DJ, Ross AG, Li YS, McManus DP. Diagnosis and management of schistosomiasis. *BMJ* 2011;**342**:d2651. http://dx.doi.org/10.1136/bmj.d2651.

4. World Health Organization. Schistosomiasis. Fact Sheet No. 115. Geneva: WHO; 2010. Available at: http://www.who.int/mediacentre/factsheet/fs115/en/index.html (accessed October 2010).

5. Gryseels BP, Clerinx J, Kestens L. Human schistosomiasis. *Lancet* 2006;**368**: 1106–18.

6. Van Der Werf MJ, De Vlas SJ. Morbidity and infection with schistosomes or soil-transmitted helminths. *Acta Tropica* 2001;**86**:1–3.

7. Bica I, Hamer DH, Stadecker MJ. Hepatic schistosomiasis. *Infect Dis Clin North Am* 2000;**14**:583–604.

8. Abdel-Wahab MF, Esmat G, Ramzy I, Fouad R, Abdel-Rahman M, Yosery A, Narooz S, Strickland GT. Schistosoma haematobium infection in Egyptian schoolchildren: demonstration of both hepatic and urinary tract morbidity by ultrasonography. *Trans R Soc Trop Med Hyg* 1992;**86**(4):406–9.

9. Weber G, Borer A, Zirkin HJ, Riesenberg K, Alkan M. Schistosomiasis presenting as acute appendicitis in a traveler. *J Travel Med* 1998;**5**:147–8.

10. Jenkins-Holick DS, Kaul TL. Schistosomiasis. *Urol Nurs* 2013;**33**:163–70.

11. Ata AA, el-Raziky SH, el-Hawey AM, Rafla H. A clinicopathological study of schistosomal colonic polyposis and their pathogenesis. *J Egypt Med Assoc* 1970;**53**:762–72.

12. Betson M, Sousa-Figueiredo JC, Rowell C, Kabatereine NB, Stothard JR. Intestinal schistosomiasis in mothers and young children in Uganda: investigation of field-applicable markers of bowel morbidity. *Am J Trop Med Hyg* 2010;**83**:1048–55.

13. Elbaz T, Esmat G. Hepatic and intestinal schistosomiasis: review. *Journal of Advanced Research* 2013;**4**:445–52.

14. Chen MG. Relative distribution of Schistosoma japonicum eggs in the intestine of man: a subject of inconsistency. *Acta Trop* 1991;**48**:163–71.

15. Strickland GT. Gastrointestinal manifestations of schistosomiasis. *Gut* 1994;**35**:1334–7.

16. Ismail MM, Attia MM, el-Badawy AA, Farghaly AM, Husein MH, Metwally A. Treatment of schistosomiasis with praziquantel among school children. *J Egypt Soc Parasitol* 1994;**24**:487–94.

17. Delgado J, Delgado B, Sztarkier I, Baer A, Depsames R. Schistosomal rectal polyp—an unusual cause of rectal bleeding. *Isr Med Assoc J* 2004;**6**:114–5.

18. Mostafa I. Schistosomal colonic polyposis. *Gastrointest Endosc* 1997;**46**:584–7.

19. Strickland GT, Merritt W, El-Sahly A, Abdel-Wahab F. Clinical characteristics and response to therapy in Egyptian children heavily infected with Schistosoma mansoni. *J Infect Dis* 1982;**146**:20–9.

20. Nebel OT, el-Masry NA, Castell DO, Farid Z, Fornes MF, Sparks HA. Schistosomal disease of the colon: a reversible form of polyposis. *Gastroenterology* 1974;**67**:939–43.

21. Barsoum H. Cancer in Egypt: its incidence and clinical forms. *Acta Unio Int Contra Cancrum* 1953;**9**:241–50.

22. Liu W, Zeng HZ, Wang QM, Yi H, Mou Y, Wu CC, Hu B, Tang CW. Schistosomiasis combined with colorectal carcinoma diagnosed based on endoscopic findings and clinicopathological characteristics: a report on 32 cases. *Asian Pac J Cancer Prev* 2013;**14**(8):4839–42.

23. Madavo CaH H. Schistosomiasis of the appendix. *J R Soc Med* 2006;**99**:473–4.

24. Al-Waheeb S, Al-Murshed M, Dashti F, Hira PR, Al-Sarraf L. Disseminated peritoneal Schistosoma japonicum: a case report and review of the pathological manifestations of the helminth. *Ann Saudi Med* 2009;**29**:149–52.

25. Schistosomiasis Research Group. Schistosome egg granuloma in human liver. Department of Pathology, University of Cambridge; 2010. Available at: http://www.path.cam.ac.uk/~schisto/schistosoma/schisto_pathology_granuloma.html (Accessed June 16, 2014).

26. Boros DL. T helper cell populations, cytokine dynamics, and pathology of the schistosome egg granuloma. *Microbes Infect* 1999;**1**:511–6.

27. Andrade ZA. Schistosomiasis and liver fibrosis. *Parasite Immunol* 2009;**31**: 656–63.

28. Hussein MR. Mucocutaneous Splendore–Hoeppli phenomenon. *J Cutan Pathol* 2008;**35**:979–88.

29. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. *Lancet* 2014;**383**(9936):2253–64. http://dx.doi.org/10.1016/S0140-6736(13)61949-2. Epub 2014 Apr 1. Review.

30. Butrous G, Ghofrani HA, Grimminger F. Pulmonary vascular disease in the developing world. *Circulation* 2008;**118**:1758–66.

31. Carvalho VT, Barbosa MM, Nunes MC, Cardoso YS, de Sá Filho IM, Oliveira FR, Antunes CM, Lambertucci JR. Early right cardiac dysfunction in patients with schistosomiasis mansoni. *Echocardiography* 2011;**28**(3):261–7. http://dx.doi.org/10.1111/j.1540-8175.2010.01330.x. Epub 2011 Feb 17.

32. Brito TD, Nussenzveig I, Carneiro CR, Silva AM. Schistosoma mansoni associated glomerulopathy. *Rev Inst Med Trop São Paulo* 1999;**41**:269–72.

33. Rodrigues VL, Otoni A, Voieta I, Antunes CM, Lambertucci JR. Glomerulonephritis in schistosomiasis mansoni: a time to reappraise. *Rev Soc Bras Med Trop* 2010;**43**:638–42.

34. Leite LA, Domingues AL, Lopes EP, Ferreira RD, Filho Pimenta AD, da Fonseca CS, Dos Santos BS, Lima VL. Relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis. *Rev Bras Hematol Hemoter* 2013;**35**(5):332–6.

35. Shimada M, Kirinoki M, Shimizu K, Kato-Hayashi N, Chigusa Y, Kitikoon V, Pongsasakulchoti P, Matsuda H. Characteristics of granuloma formation and liver fibrosis in murine schistosomiasis mekongi: a morphological comparison between Schistosoma mekongi and S. japonicum infection. *Parasitology* 2010;**137**(12):1781–9. http://dx.doi.org/10.1017/S0031182010000806. Epub 2010 Jun 21.

36. Monchy D, Dumurgier C, Heng TK, Hong K, Khun H, Hou SV, Sok KE, Huerre MR. Histology of liver lesions due to Schistosoma mekongi. About six cases with severe portal hypertension operated in Cambodia. *Bull Soc Pathol Exot* 2006;**99**(5):359–64.

37. Ohmae H, Sinuon M, Kirinoki M, Matsumoto J, Chigusa Y, Socheat D, Matsuda H. Schistosomiasis mekongi: from discovery to control. *Parasitol Int* 2004;**53**(2):135–42. Review.

38. Keang H, Odermatt P, Odermatt-Biays S, Cheam S, Degremont A, Hatz C. Liver morbidity due to Schistosoma mekongi in Cambodia after seven rounds of mass drug administration. *Trans R Soc Trop Med Hyg* 2007;**101**:759–65.

39. Stich AH, Biays S, Odermatt P, Men C, Saem C, Sokha K, Ly CS, Legros P, Philips M, Lormand JD, Tanner M. Foci of Schistosomiasis mekongi, Northern Cambodia: II. Distribution of infection and morbidity. *Trop Med Int Health* 1999;**4**(10):674–85.

40. Fallon PG, Richardson EJ, McKenzie GJ, McKenzie AN. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J Immunol* 2000;**164**:2585–91.

41. Hams E, Aviello G, Fallon PG. The Schistosoma granuloma: friend or foe? *Front Immunol* 2013;**4**:89. http://dx.doi.org/10.3389/fimmu.2013.00089. eCollection 2013.

42. Anthony BJ, Ramm GA, McManus DP. Role of resident liver cells in the pathogenesis of schistosomiasis. *Trends Parasitol* 2012;**28**:572–9.

43. Bartley PB, Ramm GA, Jones MK, Ruddell RG, Li Y, McManus DP. A contributory role for activated hepatic stellate cells in the dynamics of Schistosoma japonicum egg-induced fibrosis. *Int J Parasitol* 2006;**36**:993–1001.

44. Chang D, Ramalho LN, Ramalho FS, Martinelli AL, Zucoloto S. Hepatic stellate cells in human schistosomiasis mansoni: a comparative immunohistochemical study with liver cirrhosis. *Acta Trop* 2006;**97**:318–23.

45. Anthony BJ, James KR, Gobert GN, Ramm GA, McManus DP. Schistosoma japonicum eggs induce a proinflammatory, anti-fibrogenic phenotype in hepatic stellate cells. *PLoS One* 2013;**8**(6):e68479. Print 2013.

46. Symmers W. Note of a new form of liver cirrhosis due to the presence of ova of Bilharzia haematobium. *J Pathol Bacteriol* 1904;**9**:237–9.

47. Hashem M. Etiology and pathogenesis of endemic form of hepatosplenomegaly: Egyptian splenomegaly. *J R Egypt Med Ass* 1947;**30**:48–79.

48. Hou XY, Ellis MK, McManus DP, Wang YY, Li SD, Williams GM, Li YS. Diagnostic value of non-invasive bio-markers for stage-specific diagnosis of hepatic fibrosis in patients with advanced schistosomiasis japonica. *Int J Parasitol* 2011;**41**(3–4):325–32. http://dx.doi.org/10.1016/j.ijpara.2010.10.007. Epub 2010 Dec 8.

49. Lambertucci JR. Revisiting the concept of hepatosplenic schistosomiasis and its challenges using traditional and new tools. *Rev Soc Bras Med Trop* 2014;**47**:130–6.

50. Marinho CC, Voieta I, Azeredo LM, Nishi MP, Batista TS, Pereira AC, Serufo JC, Queiroz LC, Ruiz-Guevara R, Antunes CM, Prata A, Lambertucci JR. Clinical versus ultrasound examination in the evaluation of hepatosplenic schistosomiasis mansoni in endemic areas. *Mem Inst Oswaldo Cruz* 2006;**101**(Suppl 1):317–21.

51. Pinto-Silva RA, Queiroz LC, Azeredo LM, Silva LC, Lambertucci JR. Ultrasound in schistosomiasis mansoni. *Mem Inst Oswaldo Cruz* 2010;**105**:479–84.

52. Gerspacher-Lara R, Pinto-Silva RA, Rayes AA, Drummond SC, Lambertucci J. Ultrasonography of periportal fibrosis in schistosomiasis mansoni in Brazil. *Trans R Soc Trop Med Hyg* 1997;**91**:307–9.

53. Martins MJ, Pinto-Silva RA, Serufo JC, Rayes AA, Damasceno MP, Martins ML, Santos AP, Drummond SC, Bezerra MA, Lambertucci JR. Morbidity of schistosomiasis in an endemic area of the northeast of the state of Minas Gerais in Brazil: a clinical and sonographic study. *Mem Inst Oswaldo Cruz* 1998;**93**(Suppl 1):243–4.

54. Hussain S, Hawass ND, Zaidi AJ. Ultrasonographic diagnosis of schistosomal periportal fibrosis. *J Ultrasound Med* 1984;**3**:449–52.

55. Cerri GG, Alves VA, Magalhães A. Hepatosplenic schistosomiasis mansoni: ultrasound manifestations. *Radiology* 1984;**153**:777–80.

56. Fataar S, Bassiony H, Satyanath S, Vassileva J, Hanna RM. Characteristic sonographic features of schistosomal periportal fibrosis. *Am J Roentgenol* 1984;**143**:69–71.

57. Chigusa Y, Otake H, Ohmae H, Kirinoki M, Ilagan EJ, Barzaga NG, Kawabata M, Hayashi M, Matsuda H. Determination of the period for establishment of a liver network echogenic pattern in Schistosoma japonicum infection. *Parasitol Int* 2006;**55**(1):33–7. Epub 2005 Oct 10.

58. 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. *Parasitol Int* 2003;**52**:385–93.

59. Hammerman AM, Kotner LM, Doyle TB. Periportal contrast enhancement on CT scans of the liver. *Am J Roentgenol* 1991;**156**:313–5.

60. Araki T, Hayakawa K, Okada J, Hayashi S, Uchiyama G, Yamada K. Hepatic schistosomiasis japonica identified by CT. *Radiology* 1985;**157**:757–60.

61. Lambertucci JR, Silva LC, Andrade LM, Queiroz LC, Pinto-Silva RA. Magnetic resonance imaging and ultrasound in hepatosplenic schistosomiasis mansoni. *Rev Soc Bras Med Trop* 2004;**37**:333–7.

62. Lee JK. Computed body tomography with MRI correlation. Lippincott Williams & Wilkins; 2006.

63. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* 2005;**115**:209–18.

64. Marinho CC, Bretas T, Voieta I, Queiroz LC, Ruiz-Guevara R, Teixeira AL, Antunes CM, Prata A, Lambertucci JR. Serum hyaluronan and collagen IV as non-invasive markers of liver fibrosis in patients from an endemic area for schistosomiasis mansoni: a field-based study in Brazil. *Mem Inst Oswaldo Cruz* 2010;**105**(4):471–8.

65. Domingues AL, Medeiros TB, Lopes EP. Ultrasound versus biological markers in the evaluation of periportal fibrosis in human Schistosoma mansoni. *Mem Inst Oswaldo Cruz* 2011;**106**:802–7.

66. Rojkind M, González E. An improved method for determining specific radio-activities of proline-14C and hydroxyproline-14C in collagen and in noncollagenous proteins. *Anal Biochem* 1974;**57**:1–7.

67. Bienkowski R, Cowan M, McDonald J, Crystal R. Degradation of newly synthesized collagen. *J Biol Chem* 1978;**253**:4356–63.

68. McCullough A, Stassen W, Wiesner R, Czaja A. Serum type III procollagen peptide concentrations in severe chronic active hepatitis: relationship to cirrhosis and disease activity. *Hepatology* 1987;**7**:49–54.

69. Bruckner P, Bächinger HP, Engel J, Timpl R. Three conformationally distinct domains in the amino-terminal segment of type III procollagen and its rapid triple helix ⇌ coil transition. *Eur J Biochem* 1978;**90**:595–603.

70. Friedman S. Molecular mechanisms of hepatic fibrosis and principles of therapy. *J Gastroenterol* 1997;**32**:424–30.

71. Zwingenberger K, Harms G, Feldmeier H, Müller O, Steiner A, Bienzle U. Liver involvement in human schistosomiasis mansoni: regression of immunological and biochemical disease markers after specific treatment. *Acta Trop* 1988;**45**:263–75.

72. Ricard-Blum S, Hartmann DJ, Grenard P, Ravaoalimalala VE, Boisier P, Esterre P. Relationships between several markers of extracellular matrix turn-over and ultrasonography in human schistosomiasis mansoni. *Am J Trop Med Hyg* 1999;**60**:658–63.

73. Shahin M, Schuppan D, Waldherr R, Risteli J, Risteli L, Savolainen ER, Oesterling C, Abdel Rahman HM, el Sahly AM, Abdel Razek SM, et al. Serum procollagen peptides and collagen type VI for the assessment of activity and degree of hepatic fibrosis in schistosomiasis and alcoholic liver disease. *Hepatology* 1992;**15**(4):637–44.

74. Kardorff R, Gabone RM, Mugashe C, Obiga D, Ramarokoto CE, Mahlert C, Spannbrucker N, Lang A, Günzler V, Gryseels B, Ehrich JH, Doehring E. Schistosoma mansoni-related morbidity on Ukerewe Island, Tanzania: clinical, ultrasonographical and biochemical parameters. *Trop Med Int Health* 1997;**2**(3):230–9.

75. Kardorff R, Mugashe C, Gabone RM, Mahlert C, Doehring E. Diagnostic value of connective tissue metabolites in *Schistosoma mansoni* related liver disease. *Acta Trop* 1999;**73**:153–64.

76. Wyszomirska RM, Nishimura NF, Almeida JR, Yamanaka A, Soares EC. High serum laminin and type IV collagen levels in schistosomiasis mansoni. *Arq Gastroenterol* 2005;**42**:221–5.

77. Wyszomirska RM, Lacet Cda C, Tenório LR, Nishimura NF, Mesquita MA, Neto JB, Oliveira F, Balwani Mdo C, Almeida JR, Soares EC. Decrease of type IV collagen and TIMP-1 serum levels after splenectomy in patients with schistosomiasis mansoni. *Acta Trop* 2006;**97**(3):301–8. Epub 2006 Jan 30.

78. Lindqvist U, Laurent TC. Serum hyaluronan and aminoterminal propeptide of type III procollagen: variation with age. *Scand J Clin Lab Invest* 1992;**52**:613–21.

79. Guechot J, Loria A, Serfaty L, Giral P, Giboudeau J, Poupon R. Serum hyaluronan as a marker of liver fibrosis in chronic viral hepatitis C: effect of alpha-interferon therapy. *J Hepatol* 1995;**22**:22–6.

80. Grigorescu M. Noninvasive biochemical markers of liver fibrosis. *J Gastrointestin Liver Dis* 2006;**15**:149–59.

81. Parés A, Deulofeu R, Giménez A, Caballería L, Bruguera M, Caballería J, Ballesta AM, Rodés J. Serum hyaluronate reflects hepatic fibrogenesis in alcoholic liver disease and is useful as a marker of fibrosis. *Hepatology* 1996;**24**(6):1399–403.

82. Wong VS, Hughes V, Trull A, Wight DG, Petrik J, Alexander GJ. Serum hyaluronic acid is a useful marker of liver fibrosis in chronic hepatitis C virus infection. *J Viral Hepat* 1998;**5**:187–92.

83. Pontinha N, Pessegueiro H, Barros H. Serum hyaluronan as a marker of liver fibrosis in asymptomatic chronic viral hepatitis B. *Scand J Clin Lab Invest* 1999;**59**:343–7.

84. Santos VN, Leite-Mór MM, Kondo M, Martins JR, Nader H, Lanzoni VP, Parise ER. Serum laminin, type IV collagen and hyaluronan as fibrosis markers in non-alcoholic fatty liver disease. *Braz J Med Biol Res* 2005;**38**(5):747–53. Epub 2005 May 25.

85. Pascal M, Abdallahi OM, Elwali NE, Mergani A, Qurashi MA, Magzoub M, de Reggi M, Gharib B. Hyaluronate levels and markers of oxidative stress in the serum of Sudanese subjects at risk of infection with Schistosoma mansoni. *Trans R Soc Trop Med Hyg* 2000;**94**(1):66–70.

86. Kopke-Aguiar LA, Martins JR, Passerotti CC, Toledo CF, Nader HB, Borges DR. Serum hyaluronic acid as a comprehensive marker to assess severity of liver disease in schistosomiasis. *Acta Trop* 2002;**84**:117–26.

87. Silva CC, Domingues AL, Lopes EP, Morais CN, Santos RB, Luna CF, Nader HB, Martins JR. Schistosomiasis mansoni: ultrasound-evaluated hepatic fibrosis and serum concentrations of hyaluronic acid. *Ann Trop Med Parasitol* 2011;**105**(3):233–9. http://dx.doi.org/10.1179/136485911X12987676649629.

89. Saitou Y, Shiraki K, Yamanaka Y, Yamaguchi Y, Kawakita T, Yamamoto N, Sugimoto K, Murata K, Nakano T. Noninvasive estimation of liver fibrosis and response to interferon therapy by a serum fibrogenesis marker, YKL-40, in patients with HCV-associated liver disease. *World J Gastroenterol* 2005;**11**:476–81.

90. Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem* 1993;**268**(34):25803–10.

91. Hu B, Trinh K, Figueira WF, Price PA. Isolation and sequence of a novel human chondrocyte protein related to mammalian members of the chitinase protein family. *J Biol Chem* 1996;**271**:19415–20.

92. Zheng M, Wei-Min C, Jun-Kang Z, Shao-Ming Z, Rong-Hua L. Determination of serum levels of YKL-40 and hyaluronic acid in patients with hepatic fibrosis due to schistosomiasis japonica and appraisal of their clinical value. *Acta Trop* 2005;**96**:148–52.

93. Kelleher TB, Afdhal N. Noninvasive assessment of liver fibrosis. *Clin Liver Dis* 2005;**9**:667–83. vii.

94. Murawaki Y, Ikuta Y, Okamoto K, Koda M, Kawasaki H. Serum matrix metalloproteinase-3 (stromelysin-1) concentration in patients with chronic liver disease. *J Hepatol* 1999;**31**:474–81.

95. Murawaki Y, Yamada S, Ikuta Y, Kawasaki H. Clinical usefulness of serum matrix metalloproteinase-2 concentration in patients with chronic viral liver disease. *J Hepatol* 1999;**30**:1090–8.

96. Murawaki Y, Ikuta Y, Idobe Y, Kawasaki H. Serum matrix metalloproteinase-1 in patients with chronic viral hepatitis. *J Gastroenterol Hepatol* 1999;**14**:138–45.

97. Boeker KH, Haberkorn CI, Michels D, Flemming P, Manns MP, Lichtinghagen R. Diagnostic potential of circulating TIMP-1 and MMP-2 as markers of liver fibrosis in patients with chronic hepatitis C. *Clin Chim Acta* 2002;**316**:71–81.

98. Walsh KM, Timms P, Campbell S, MacSween RN, Morris AJ. Plasma levels of matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of metalloproteinases -1 and -2 (TIMP-1 and TIMP-2) as noninvasive markers of liver disease in chronic hepatitis C: comparison using ROC analysis. *Dig Dis Sci* 1999;**44**:624–30.

99. Fabre V, Wu H, PondTor S, et al. Tissue inhibitor of matrix-metalloprotease-1 predicts risk of hepatic fibrosis in human *Schistosoma japonicum* infection. *J Infect Dis* 2011;**203**:707–14.

100. Burchard GD, Guissé-Sow F, Diop M, Ly A, Lanuit R, Gryseels B, Gressner AM. Schistosoma mansoni infection in a recently exposed community in Senegal: lack of correlation between liver morphology in ultrasound and connective tissue metabolites in serum. *Trop Med Int Health* 1998;**3**(3):234–41.

101. Tanabe M, Sekiguchi T, Kaneko N, Kobayashi S, Takeuchi T, Coutinho A, Tateno S, Maruyama K, Okazaki I. Elevation of laminin and beta-subunit of prolyl 4-hydroxylase in the sera of human subjects with Schistosomiasis mansoni. *Jpn J Exp Med* 1989;**59**(3):109–19.

102. Parise ER, Rosa H. Serum laminin in hepatic schistosomiasis. *Trans R Soc Trop Med Hyg* 1992;**86**(2):179–81.

103. Nelson D, Gonzalez-Peralta R, Qian K, et al. Transforming growth factor-beta 1 in chronic hepatitis C. *J Viral Hepat* 1997;**4**:29–35.

104. Magalhães A, Miranda DG, Miranda RG, Araújo MI, Jesus AA, Silva A, Santana LB, Pearce E, Carvalho EM, Jesus AR. Cytokine profile associated with human chronic schistosomiasis mansoni. *Mem Inst Oswaldo Cruz* 2004;**99**(5 Suppl 1):21–6. Epub 2004 Oct 13.

105. Brandt CT, Rino M, Pitta MG, Muniz JS, Silveira Dde O, Castro CM. Evaluation of the cytokines IL-10 and IL-13 as mediators in the progression of Symmers fibrosis in patients with hepatosplenic schistosomiasis mansoni. *Rev Col Bras Cir* 2010;**37**(5):333–7.

106. Arroyo JD, Chevillet JR, Kroh EM, et al. Argonaute 2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci U S A* 2011.

107. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol* 2011;**13**(4):423–33. http://dx.doi.org/10.1038/ncb2210. Epub 2011 Mar 20.

108. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007;**9**:654–9.

109. Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet* 2011;**12**:861–74.

110. Gandhi R, Healy B, Gholipour T, et al. Circulating MicroRNAs as biomarkers for disease staging in multiple sclerosis. *Ann Neurol* 2013;**73**:729–40.

111. Rani S, Gately K, Crown J, O'Byrne K, O'Driscoll L. Global analysis of serum microRNAs as potential biomarkers for lung adenocarcinoma. *Cancer Biol Ther* 2013;**14**(12):1104–12. http://dx.doi.org/10.4161/cbt.26370. Epub 2013 Sep 4.

112. Brase JC, Wuttig D, Kuner R, Sültmann H. Serum microRNAs as non-invasive biomarkers for cancer. *Mol Cancer* 2010;**9**:306.

113. Cermelli S, Ruggieri A, Marrero JA, Ioannou GN, Beretta L. Circulating micro-RNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. *PLoS One* 2011;**6**:23.

114. Roderburg C, Mollnow T, Bongaerts B, et al. Micro-RNA profiling in human serum reveals compartment-specific roles of miR-571 and miR-652 in liver cirrhosis. *PLoS One* 2012;**7**:e32999.

115. Shrivastava S, Petrone J, Steele R, Lauer GM, Di Bisceglie AM, Ray RB. Up-regulation of circulating miR-20a is correlated with hepatitis C virus-mediated liver disease progression. *Hepatology* 2013;**58**(3):863–71. http://dx.doi.org/10.1002/hep.26296. Epub 2013 Jul 23.

116. Murakami Y, Toyoda H, Tanahashi T, Tanaka J, Kumada T, Yoshioka Y, et al. Comprehensive miRNA expression analysis in peripheral blood can diagnose liver disease. *PLoS One* 2012;**7**:e48366.

117. Wang Z, Xue X, Sun J, Luo R, Xu X, Jiang Y, Zhang Q, Pan W. An "in-depth" description of the small non-coding RNA population of Schistosoma japonicum schistosomulum. *PLoS Negl Trop Dis* 2010;**4**(2):e596. http://dx.doi.org/10.1371/journal.pntd.0000596.

118. Madavo C, Hurriez H. Schistosomiasis of the appendix. *J R Soc Med* 2006;**99**(9):473–4.

119. de Souza Gomes M, Muniyappa MK, Carvalho SG, Guerra-Sa R, Spillane C. Genome-wide identification of novel microRNAs and their target genes in the human parasite Schistosoma mansoni. *Genomics* 2011;**98**(2):96–111. http://dx.doi.org/10.1016/j.ygeno.2011.05.007. Epub 2011 May 26.

120. Simoes MC, Lee J, Djikeng A, et al. Identification of *Schistosoma mansoni* microRNAs. *BMC Genomics* 2011;**12**:1471–2164.

121. Cai P, Hou N, Piao X, Liu S, Liu H, Yang F, et al. Profiles of small non-coding RNAs in *Schistosoma japonicum* during development. *PLoS Negl Trop Dis*

2011;**5**(8):e1256. http://dx.doi.org/10.1371/journal.pntd.0001256. Epub 2011 Aug 2.

122. Cai P, Piao X, Hao L, Liu S, Hou N, Wang H, Chen Q. A deep analysis of the small non-coding RNA population in *Schistosoma japonicum* eggs. *PLoS One* 2013;**8**(5):e64003. http://dx.doi.org/10.1371/journal.pone.0064003. Print 2013.

123. Cheng G, Luo R, Hu C, Cao J, Jin Y. Deep sequencing-based identification of pathogen-specific microRNAs in the plasma of rabbits infected with *Schistosoma japonicum*. *Parasitology* 2013;**140**:1751–6.

124. Han H, Peng J, Hong Y, Zhang M, Han Y, Liu D, Fu Z, Shi Y, Xu J, Tao J, Lin J. MicroRNA expression profile in different tissues of BALB/c mice in the early phase of *Schistosoma japonicum* infection. *Mol Biochem Parasitol* 2013;**188**:1–9.

125. Cai P, Piao X, Liu S, Hou N, Wang H, Chen Q. MicroRNA-gene expression network in murine liver during *Schistosoma japonicum* infection. *PLoS One* 2013;**8**(6):e67037. http://dx.doi.org/10.1371/journal.pone.0067037. Print 2013.

126. Eboumbou C, Steghens JP, Abdallahi OM, Mirghani A, Gallian P, van Kappel A, et al. Circulating markers of oxidative stress and liver fibrosis in Sudanese subjects at risk of schistosomiasis and hepatitis. *Acta tropica* 2005;**94**:99–106.

127. Guangjin S, Mingdao J, Qiyang L, Hui X, Jiangming H, Xiaomei Y. Study on histopathology, ultrasonography and some special serum enzymes and collagens for 38 advanced patients of schistosomiasis japonica. *Acta Trop* 2002;**82**:235–46.

128. Li YS, Sleigh AC, Ross AG, Li Y, Williams GM, Tanner M, McManus DP. Two-year impact of praziquantel treatment for *Schistosoma japonicum* infection in China: re-infection, subclinical disease and fibrosis marker measurements. *Trans R Soc Trop Med Hyg* 2000;**94**:191–7.