

## Ultrasound versus biological markers in the evaluation of periportal fibrosis in human *Schistosoma mansoni*

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*In this paper, the authors review the literature and share their experience of the principal biological markers of fibrosis for the evaluation of periportal fibrosis (PPF) caused by mansoni schistosomiasis. These biological markers are compared to diagnostic ultrasound (US) scans as means of grading PPF. We also review procollagen type I and III, collagen type IV, laminin, hyaluronic acid (HA), immunoglobulin G, platelets, aspartate aminotransferase to platelet ratio index (APRI) and gamma-glutamyl transpeptidase as markers of the disease. Although there are several good markers for evaluating PPF and portal hypertension, such as HA, platelets or APRI, none can yet replace US. These markers may, however, be used to identify patients at greater risk of developing advanced disease in endemic areas and determine who will need further care and US studies.*

Key words: schistosomiasis mansoni - ultrasound - fibrosis markers - HS schistosomiasis - liver fibrosis

The assessment of liver periportal fibrosis (PPF) provides useful information for adopting therapeutic decisions in patients infected with *Schistosoma mansoni*. It is also an important approach in the hospital and in the field for identifying the disease morbidity. While liver-wedge biopsy is the most accurate method for assessing fibrosis, it nonetheless has certain limitations and risks and needle biopsy is not sufficiently sensitive to diagnose PPF (Dimmette 1955, Maia et al. 2007). Over the last 15-20 years, especially in areas where schistosomiasis is endemic, upper abdominal ultrasound (US) has become the most useful diagnostic tool for diagnosing and quantifying PPF (Abdel-Wahab et al. 1992, Marinho et al. 2000, Richter 2000, Richter et al. 2001). However, this method is not readily available in all endemic areas because it requires both US equipment and qualified examiners. In addition, US findings show wide variation within and between observers (Gryssels et al. 2006). Using the Niamey criteria, Santos et al. (2007) demonstrated moderate to substantial intra and inter-observer reproducibility in PPF classification. This finding has led to the development of noninvasive biochemical markers of liver fibrosis. Direct markers that reflect extracellular matrix turnover are available, while indirect markers that reflect alterations in liver function also exist.

The characteristics of an ideal marker of hepatic fibrosis would include noninvasiveness, liver specificity, ease of performance, sensitivity, reproducibility and quickness (Grigorescu 2006). From a practical viewpoint, the aim of biochemical noninvasive investigation in mansoni schistosomiasis is to discriminate between patients with no fibrosis to mild fibrosis and those with mild to advanced fibrosis.

Accordingly, the aim of this paper is to relate our experiences and those of others of schistosomiasis using studies that correlate serum markers of hepatic fibrosis with different patterns of US-established PPF.

*Direct markers* - Direct markers are less useful in clinical practice, due to their costly and difficult methods.

*Procollagen type I carboxy-terminal peptide (PICP)* - PICP has been little studied in schistosomiasis. Ricard-Blum et al. (1999) showed that PICP levels were higher in infected patients than in endemic controls and that serum levels decreased with praziquantel during the first post-treatment year. Nevertheless, no correlation has been established between PICP and fibrosis US scores.

*Procollagen type III amino-terminal peptide (PII-*INP*)* - In mansoni schistosomiasis, the serum levels of PIIINP have been correlated with cases with portal hypertension and with more severe hepatic diseases.

Zwingenberger et al. (1988), while studying 82 individuals infected without liver enlargement and 20 with hepatic or hepatosplenic (HS) disease, revealed that PIIINP was elevated in patients with hepatic disease, but normal in uncomplicated cases. This same author, in 1990, monitored 23 patients with HS schistosomiasis 18 months after being treated with praziquantel and showed that PIIINP levels had returned to normal. Braga et al. (1990) found no significant difference in the serum levels of PIIINP six months before and after treatment with oxamniquine. However, it is possible that this follow-up period was insufficient. Shahin et al. (1992) observed higher serum levels of PIIINP in patients with mansoni schistosomiasis than in the control group with even higher levels in patients with advanced PPF as diagnosed by histology.

Other authors, while studying intestinal or hepatointestinal patients, did not observe any significant difference in the serum levels of PIIINP that correlated with the presence or intensity of infection (Kardorff et al. 1999), egg-positive individuals (Tanabe et al. 1989) or fibrosis scores (Kardorff et al. 1997, Burchard et al. 1998, Ricard-Blum et al. 1999).

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*Serum type VI collagen* - Kardorff et al. (1997, 1999) demonstrated that the procollagen IV peptide did not correlate with the presence or intensity of schistosomiasis infection, but it was significantly correlated with liver fibrosis, splenomegaly, portal vein dilatation and the presence of portosystemic collaterals. In the latter study, these researchers found that 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.

Wyszomirska et al. (2005, 2006) indicated a positive correlation between type IV collagen and advanced forms of the disease, with a significant reduction being observed in serum levels following splenectomy. These researchers did not observe a correlation to the grade of PPF when using US evaluation as gold-standard method.

*Laminin* - Laminin is a major noncollagenous glycoprotein of basement membranes. Laminin is synthesised by hepatic stellate cells (HSCs) and it is correlated with portal hypertension (Ricard-Blum et al. 1999).

Some field studies evaluating individuals with milder forms of schistosomiasis did not reveal a correlation between laminin and PPF fibrosis (Kardorff et al. 1997, 1999, Burchard et al. 1998, Ricard-Blum et al. 1999) and the presence or intensity of infection (Kardorff et al. 1999). However, Tanabe et al. (1989) and Ricard-Blum et al. (1999) noted that the mean value of serum laminin was significantly higher in egg-positive infected patients than in endemic controls.

Further, the serum levels of laminin were higher in HS than in hepatointestinal patients and were also higher in hepatointestinal patients than the controls (Parise & Rosa 1992).

Finally, Wyszomirska et al. (2005) showed a progressive increase of laminin in the initial stages of disease in hepatointestinal cases, as well as in advanced disease forms.

*Hyaluronic acid (HA)* - HA is a glycosaminoglycan, a component of the extracellular matrix and is synthesised by HSCs. Under normal circumstances, the endothelial cells of liver sinusoids are the sites of HA uptake and degradation. Increased HA levels are either due to decreased hepatic removal, increased production or both (Grigorescu 2006).

Several studies have examined HA serum levels in a number of chronic liver diseases and have suggested that it may be a good marker for detecting fibrosis in cirrhosis, chronic hepatitis B and C and alcoholic and nonalcoholic fatty liver disease (Guéchet et al. 1995, Parés et al. 1996, Wong et al. 1998, Pontinha et al. 1999, Santos et al. 2005).

Ricard-Blum et al. (1999), while studying patients with intestinal and HS schistosomiasis and with mild to advanced fibrosis, found higher levels of HA in more advanced forms of schistosomiasis, thereby suggesting a correlation with disease severity. In the same study, the authors reported reduced HA levels following specific treatment with praziquantel.

A reduction in serum HA level was also revealed following early treatment with praziquantel in patients with the intestinal form of the disease (Hassanein et al. 1997).

Burchard et al. (1998), studying 153 patients in Senegal with schistosomotic hepatic fibrosis, found no changes in patient levels of HA, laminin or PIIINP. A possible reason underlying these results could be that 60% of patients showed early stages of hepatic involvement with US, while only 3% presented with HS disease.

Alternately, Köpke-Aguiar et al. (2002) reported a positive correlation between the levels of HA and the intensity of PPF assessed by a receiver operator characteristic (ROC) curve that generated a cut-off that accurately differentiated the mild form from severe forms.

Pascal et al. (2000) and Eboumbou et al. (2005) showed that serum HA levels are increased in advanced forms of the disease. These reports suggested that HA could be a useful marker for evaluating disease morbidity and for monitoring fibrosis reversal following appropriate therapy.

We have assessed 122 patients with PPF and 12 schistosomotic patients without fibrosis. Serum levels of HA in the nonfibrotic group was 23.9 micrograms per litre (mcg/L), 51.9 mcg/L in the mild fibrotic group and 64.3 mcg/L in those with advanced fibrosis. In order to identify the best cut-off for patients with fibrosis, the ROC plot showed a serum value of 27.8 mcg/L with a sensitivity of 78.2% and a specificity of 83.7% (data not published).

In a previous study, of 61 patients with mansoni schistosomiasis and 16 healthy individuals, a serum HA level of 20.2 mcg/L was observed that differentiated between patients with milder PPF (patterns C+D) and those with more severe PPF (patterns E+F) with a sensitivity of 60% and a specificity of 65% (Silva et al. 2011).

Indeed, Köpke-Aguiar et al. (2002) found that an HA level of 20.0 mcg/L separated schistosomotic patients with and without portal hypertension.

Marinho et al. (2010) evaluating serum levels of HA and type IV collagen with respect to ultrasonographic patterns of PPF, showed that only the serum HA levels were capable of separating patients with mild fibrosis from those with intense fibrosis. These authors also reported a positive correlation between HA serum levels and signs of portal hypertension (using portal and splenic vein diameters).

*Indirect markers - Platelet count (PLT)* - Thrombocytopenia is a valuable marker of advanced liver disease, but many mechanisms may cause it, including hypersplenism, myelosuppression by hepatitis C, decreased thrombopoietin production and autoimmune processes (Grigorescu 2006).

A decrease in the number of platelets has been observed in chronic liver disease, especially in hepatitis C, liver cirrhosis and schistosomiasis, as the disease worsens (Souza et al. 2000, Silva et al. 2008, Kim et al. 2009, Vardar et al. 2009).

In HS schistosomiasis, hypersplenism (due to an enlarged spleen) results in thrombocytopenia and is found principally in a subgroup of patients, as a marker of portal hypertension and more advanced PPF (Maia et al. 2007). Indeed, some schistosomiasis studies that evaluated platelets and other serum markers have reported lower platelet levels in patients with more advanced fibrosis (Köpke-Aguiar et al. 2002, Al Mofarreh et al. 2003, el-Shorbagy et al. 2004).

A moderate decrease in the number of platelets is a marker of the HS disease form with portal hypertension and a PLT of approximately 130,000/mm<sup>3</sup> can accurately differentiate between schistosomotic patients with and without portal hypertension (Souza et al. 2000).

Lambertucci et al. (2007) conducted a study involving 47 patients with HS schistosomiasis and 13 with the hepatointestinal disease form. The authors correlated the different degrees of PPF with the number of platelets and found an average number of 194,646 platelets for the patients without fibrosis by US but 44,114 platelets in patients with severe fibrosis.

Köpke-Aguiar et al. (2002) found that a cut-off of 120,000 platelets revealed a 100% diagnostic efficacy in distinguishing schistosomotic patients with and without portal hypertension.

Additionally, an inverse relationship between spleen size and PLT was suggested by Correia et al. (2009b).

We have found with 122 patients with schistosomiasis that the PLT showed a significant negative correlation with the different PPF patterns. These data showed that the more advanced stages of fibrosis were associated with lower platelet counts than observed in the non-fibrosis group (257,833 ± 74,215/mm<sup>3</sup>; mild fibrosis group: 158,355 ± 79,729/mm<sup>3</sup>; severe group: 96,430 ± 45,355/mm<sup>3</sup>). The PLT ability, represented by the area under the ROC curve, to identify patients with PPF was 0.921 with the best cut-off value of 171,000/mm<sup>3</sup>, a sensitivity of 80% and a specificity of 91.7%. A value of 141,000 platelets differentiated mild from severe fibrosis with a sensitivity of 78.5% and a specificity of 60%.

*Aspartate aminotransferase to platelet ratio index (APRI)* - Combined assessment of the aspartate aminotransferase/platelet ratio (ratio of aspartate aminotransferase levels in international units per litre, as divided by the upper limit of normal x 100 and platelet count) had a high diagnostic accuracy for cirrhosis and has been used in schistosomotic patients. The APRI has the benefit of including tests used in clinical practice.

It was first studied in chronic hepatitis C (Wai et al. 2003).

In a meta-analysis involving 22 studies, an APRI of above 1.5 was positively correlated, with a sensitivity of 88% for significant fibrosis (≥ F2), whereas an index below 0.5 was positively correlated with 76% sensitivity to fibrosis exclusion. An APRI score of above 2.0 had good specificity for diagnosing cirrhosis (Shaheen & Myers 2008).

In our study of 122 patients, the APRI indicated increasing values with disease group: no fibrosis (0.26 ± 0.11), mild fibrosis (0.92 ± 0.98) and severe fibrosis (1.58 ± 1.40). The accuracy for identifying fibrosis was 0.93 and a value of 0.349 was the best cut-off, with a sensitivity of 90% and a specificity of 83.3% being observed.

Lambertucci et al. (2007) studied platelets and APRI in a study involving 47 patients with the HS form of the disease. They observed APRI values of 0.34, 1.38, 1.74 and 1.96 for patients with no fibrosis, mild, moderate and severe fibrosis, respectively. They concluded that APRI appeared to be a promising marker for evaluating PPF in mansoni schistosomiasis.

*Immunoglobulin G (IgG)* - Recent studies have shown that immunoglobulins exert a direct effect on hepatic fibrogenesis and that IgGs stimulate the proliferation of HSCs (Sebastiani & Alberti 2006).

A strong association was found between serum immunoglobulin levels and hepatic fibrosis, particularly in patients with chronic hepatitis B virus and hepatitis C virus infection and auto-immune hepatitis (Watt et al. 2004, Schmilovitz-Weiss et al. 2006, Lüth et al. 2008).

Silveira et al. (2002) reported significantly higher IgG4 levels in patients with PPF when evaluating IgG4 in schistosomotic patients with and without PPF.

Recently, our group evaluated 41 patients with schistosomotic PPF and observed higher IgG serum levels that correlated with progression of PPF intensity (Correia et al. 2009a).

With 122 patients, we observed that the mean serum level of IgG was 1,236 mg/dL (± 368) in the group with no fibrosis and was 1,338 mg/dL (± 379) and 1,647 mg/dL (± 242) in the groups with moderate and severe fibrosis, respectively. The value of 1,477 mg/dL was the best cut-off to separate mild from advanced fibrosis with a sensitivity of 78.5%, a specificity of 64.4% and an accuracy of 0.77%.

*Gama glutamyl transpeptidase (GGT)* - Some studies have identified GGT as a prognostic marker of fibrosis in chronic liver diseases (Oberti et al. 1997, Iacobellis et al. 2005) and schistosomiasis (Souza et al. 2000).

Several mechanisms have been proposed to explain the elevation of alkaline phosphatase and GGT in schistosomiasis, including parasitic load, PPF and intrahepatic hepatobiliary alterations (Coutinho 1960, Barreto 1971, Vianna et al. 1989).

Mansour et al. (1982) observed that GGT was elevated in the mild forms of the disease, due to the intense migration of eggs to the liver and formation of granulomas. These researchers also observed that the level of GGT was higher in the HS form with gastrointestinal bleeding.

Vianna et al. (1989), studying 132 patients with HS schistosomiasis, noted alterations in the intrahepatic biliary ducts, such as ductular proliferation and degeneration of the biliary ducts, following hepatic biopsy of patients with active infections.

Martins and Borges (1993) found that the mechanism underlying elevation of GGT in schistosomotic patients was different from that of alcoholic patients. The elevation was not due to mitochondrial lesions, as in the latter group, but rather was due to hepatobiliary injury.

The study of Al Mofarreh et al. (2003) suggested that elevated GGT was also due to hepatobiliary alterations.

Recently, alterations were described in the second and third order biliary branches using magnetic resonance cholangiopancreatography imaging in HS patients with elevated GGT (Brant et al. 2008).

In Tanzania, studying 439 patients, Kardorff et al. (1997) did not find any correlation of GGT with the parasitic load. However, the authors discovered higher levels in patients with more advanced PPF.

Amaral et al. (2002) evaluated 25 patients with schistosomiasis and compared GGT and US and they did not observe a positive correlation between the US portal and

periportal changes and increases in GGT. Furthermore, they did not find a positive correlation between the parasitic load and an increase in GGT enzyme activity. These researchers concluded that US was not a sensitive method for detecting the intrahepatic alterations that cause higher GGT levels.

Nevertheless, a relationship between the portal flux, as evaluated by US-doppler and elevated GGT was observed by Alves et al. (2003) in patients with the HS disease form.

We note that with 122 patients we discovered a correlation between GGT levels (GGT/NSL) and PPF with 0.84% accuracy, 74.6% sensitivity and 83.3% specificity for diagnosing PPF.

A revision of the principal biochemical markers for detection and evaluation of PPF in mansoni schistosomiasis has been undertaken with the objective of helping developing countries identify patients with more advanced forms of the disease.

US was introduced to the study of schistosomiasis over the last 15 years, being a safe, rapid, noninvasive and inexpensive technique, but, unfortunately, it is not always readily available in endemic areas.

Until now, HA appears to have been the most promising direct marker for diagnosing and evaluating the intensity of PPF in mansoni schistosomiasis. A correlation has also been identified between HA and signs of portal hypertension with reduced HA serum levels after specific treatment of schistosomiasis. The disadvantage of HA and the other direct markers is their difficult methodology that is not routinely available. One further problem of direct markers is the lack of organ specificity. There are connective tissues, with identical components, all over the body; therefore, fibrosis and fibrotic disorders in any body compartment will lead to alterations in the serum levels of these markers. These markers also cannot be used in children, due to skeletal growth or in individuals with acute inflammation.

From among the indirect markers platelets, the APRI and GGT appear to be good markers.

All of these markers (HA, platelets, APRI, GGT) showed a correlation to portal hypertension (Mansour et al. 1982, Souza et al. 2000, Köpke-Aguiar et al. 2002, Marinho et al. 2010).

In schistosomiasis, vascular lesions within the fibrotic tissue are the main factors underlying the physiological disorder (Andrade 2008). Therefore, there is a correlation between PPF and portal hypertension and we do not know exactly whether these markers are evaluating the level of portal hypertension or the level of fibrosis or both. In order to answer this question, more studies are necessary, using animal models of HS schistosomiasis, undertaking longitudinal follow-up of infected animals and comparing HS schistosomotic patients with and without portal hypertension.

The biochemical markers of hepatic fibrosis should be reliable and sensitive so as to indicate advanced lesions, however, they should also possess a prognostic value for identifying early disease.

In conclusion, there are several good biological markers of fibrosis, but as yet, we have been unable to find an

ideal marker to replace US findings in evaluating PPF in schistosomiasis. These markers could be used in the field to select the patients with more advanced lesions who will require further attention.

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