

CORRELATION BETWEEN PLATELET COUNT AND BOTH LIVER FIBROSIS AND SPLEEN DIAMETER IN PATIENTS WITH *SCHISTOSOMIASIS MANSONI*

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ABSTRACT - Context - Studies have described the correlation between platelet count and the stages of fibrosis in chronic viral hepatitis, but few publications have studied this correlation in *Schistosomiasis mansoni*. **Objective** - Therefore, this study aimed to correlate platelet count with both the periportal fibrosis pattern and spleen diameter evaluated by ultrasound exam in patients with *Schistosomiasis mansoni*. **Methods** - Patients with *Schistosomiasis mansoni* were evaluated by abdominal ultrasound by a single examiner for the determination of periportal fibrosis pattern (Niaimey classification) and spleen diameter. Platelet counts were performed in an automated cell counter. **Results** - One hundred eighty-seven patients with *Schistosomiasis mansoni* (mean age: 50.2 years) were included in the study, 114 of whom (61%) were women. Based on the Niaimey classification, the ultrasound analysis revealed that 37, 64, 64 and 22 patients exhibited patterns C, D, E and F, respectively. In these four groups, the mean number of platelets was 264, 196, 127 and 103 x 10⁹/L and mean spleen diameter was 9.2, 11.9, 14.9 and 16.2 centimeters, respectively. A reduction in platelet count was significantly associated with both the progression of the periportal fibrosis and the increase in spleen size. **Conclusion** - Platelet count in patients with *Schistosomiasis mansoni* was inversely correlated with the severity of periportal fibrosis and spleen diameter. **HEADINGS** - Liver fibrosis. Platelet count. Splenomegaly. Thrombocytopenia. Portal hypertension. *Schistosomiasis mansoni*.

INTRODUCTION

In the hepatosplenic form of *Schistosomiasis mansoni* (SM), portal hypertension occurs due to both periportal fibrosis (Symmers), which increases resistance to blood flow, and hyperflow in the splenic vein due to the splenomegaly^(13, 18, 24). Indeed, a correlation has been described between the intensity of periportal fibrosis and pressure levels in the portal vein, which can lead to bleeding through esophageal varices; moreover, an association has been described between the presence of esophageal varices and spleen diameter^(1, 21).

Liver biopsy⁽⁷⁾, an ultrasound examination of the abdomen^(3, 8), serum markers^(5, 10, 16, 22), platelet count^(4, 12) and, more recently, liver stiffness (elastography) measurement⁽⁹⁾ have been used to assess the fibrosis that develops in chronic liver disease. With SM, the liver-wedge biopsy is the most accurate method for measuring periportal fibrosis, but implies the use of laparotomy. Nevertheless, the needle biopsy does not always represent the true histopathological

condition, as fibrosis varies in intensity throughout the hepatic parenchyma⁽⁷⁾.

Ultrasound is an important tool in the diagnosis of the alterations related to SM, allowing the identification of the enlargement of the left lobe of the liver, periportal thickening and the increase in the length of the spleen^(3, 8, 20). The Niaimey ultrasound classification allows determining the pattern of periportal fibrosis, which is used to assess its intensity with satisfactory accuracy and reliability^(8, 20, 26). However, this method requires the availability of ultrasound equipment and trained operators. Thus, simpler methods are desirable.

Due to the drawbacks of liver biopsies and the large-scale use of ultrasound, substances involved in the production or degradation of collagen have recently been investigated for the determination of fibrosis in liver disease^(10, 12, 19, 22, 27). Stellate liver cells (Kupffer cells), which are mediators of liver fibrosis, are activated by tumor necrosis factor-alpha (TNF- α), platelet-derived growth factor (PDGF) and fibrogenic cytokines, such as TGF- β , IL-13, angiotensin II and

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leptin^(2, 14, 15). Besides the proteins responsible for fibrogenesis others stemming from the degradation process of the extracellular matrix have been studied in the evaluation of fibrosis, such as hyaluronic acid, laminin and type IV collagen^(10, 16, 19, 27). Likewise, the number of platelets is reported to be inversely correlated with the intensity of periportal fibrosis due to the sequestering of platelets in the splenic parenchyma stemming from splenomegaly^(5, 12, 17, 23).

Considering the scarcity of accurate data regarding the measurement of fibrosis using serum markers in patients with SM, the aim of this study was to correlate platelet count with both the periportal fibrosis pattern and spleen diameter as evaluated through an ultrasound exam.

METHODS

Patients

A descriptive, cross-sectional study was carried out involving patients with SM who consecutively underwent an ultrasound exam over an eight-month period at the Gastroenterology Section of the Hospital das Clínicas of the Universidade Federal de Pernambuco (UFPE), Brazil. The diagnosis of SM was based on their clinical history of contact with contaminated water and/or reports of prior treatment for SM and ultrasound examination of the upper abdomen showing periportal fibrosis.

Male and female patients aged 18 years or older with SM diagnosis who had not undergone splenectomy were included. The following were the exclusion criteria: presence of markers for hepatitis B and C (anti-HBc and anti-HCV); alcohol intake >210 g/week; and ultrasound evidence of other liver disease, as expressed by the presence of steatosis or fine fibrosis diffused throughout the parenchyma.

Methods

Laboratory exams were performed at the Central Laboratory of the aforementioned hospital. Platelet counts were performed using an automated cell counter (Cell-Dyn 3000), and anti-HBc and anti-HCV were detected using immune enzyme assays (Abbott).

Ultrasound exams were performed by a single operator (ALCD) using the Aloka SSD-500 device with convex transducer a 3.5 MHz for the evaluation of the liver and spleen. Periportal fibrosis was evaluated based on the Niamey classification, which has six pre-established patterns of fibrosis intensity, ranging from Pattern A (normal) to Pattern F (very advanced fibrosis)^(20, 26).

Statistical analysis

Statistical analysis was performed using the SPSS 12 and Excel 2000 programs. The variables were presented in tables and graphs, with the data expressed as mean and standard deviation values. The Kolmogorov-Smirnov test was used to determine the distribution of the data (parametric or non-parametric). ANOVA followed by Levene's test was used for the determination of equal variances in the analysis of the variables according to the fibrosis pattern. As heterogeneity

was demonstrated, Tamhane's test was used. Pearson's correlation coefficients were calculated to determine correlations between the platelets count and spleen diameter. The level of significance was set to 5% ($P < 0.05$) for all statistical tests.

RESULTS

Among the 238 patients with SM who underwent an ultrasound exam and exhibited periportal fibrosis, 51 (21%) of whom presented exclusion criteria. Thus, 187 patients were included in the study, 114 (61%) of whom were female and 73 (39%) were male. Mean age was 50.2 years (22 to 77 years).

Table 1 exhibit the distribution of the patients according to the fibrosis pattern (Niamey classification), mean platelet count and spleen diameter.

Table 1. Mean platelet count ($\times 10^9/L$) and spleen diameter (cm) according to periportal fibrosis pattern (Niamey classification) in 187 patients with *Schistosomiasis mansoni*

N	Fibrosis	Platelets count ($\times 10^9/L$)	Spleen diameter (cm)
37	C	264 \pm 71 (150 to 493)	9.2 \pm 1.0 (7.0 to 12.5)
64	D	196 \pm 69 (47 to 402)	11.9 \pm 2.3 (8.8 to 20.9)
64	E	127 \pm 39 (20 to 204)	14.9 \pm 1.7 (10.0 to 21.0)
22	F	103 \pm 44 (30 to 197)	16.2 \pm 1.8 (13.3 to 19.1)

Figure 1 displays the distribution of the mean platelets counts according to the fibrosis pattern evaluated using ultrasound in the 187 patients (ANOVA $F = 57.26$; $P < 0.001$). Tamhane's test revealed significant differences in the platelet count between the different fibrosis patterns ($P < 0.001$), except between Patterns E and F ($P = 0.203$).

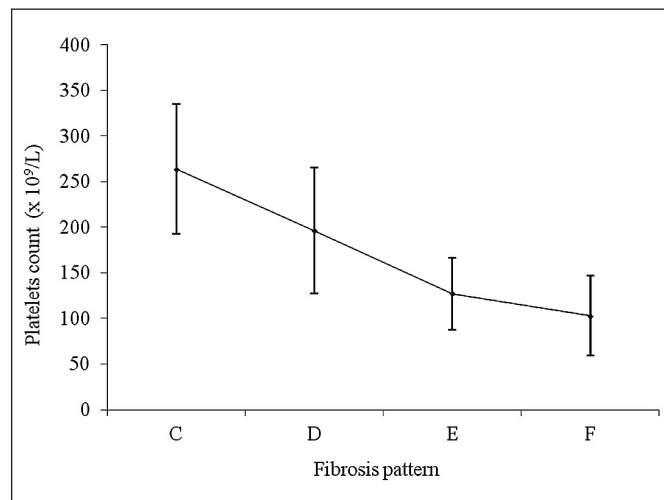


FIGURE 1. Distribution of mean platelet count ($\times 10^9/L$) according to periportal fibrosis pattern (Niamey classification) in 187 patients with *Schistosomiasis mansoni* (ANOVA $F = 57.26$; $P < 0.001$)

Figure 2 displays the distribution of the mean spleen diameter (in cm) according to the fibrosis pattern evaluated using ultrasound in the 187 patients (ANOVA $F = 108.53$; $P < 0.001$). Tamhane's test revealed significant differences in mean spleen diameter between Patterns C and D and Patterns D and E of fibrosis ($P < 0.001$), and between Patterns E and F ($P = 0.037$).

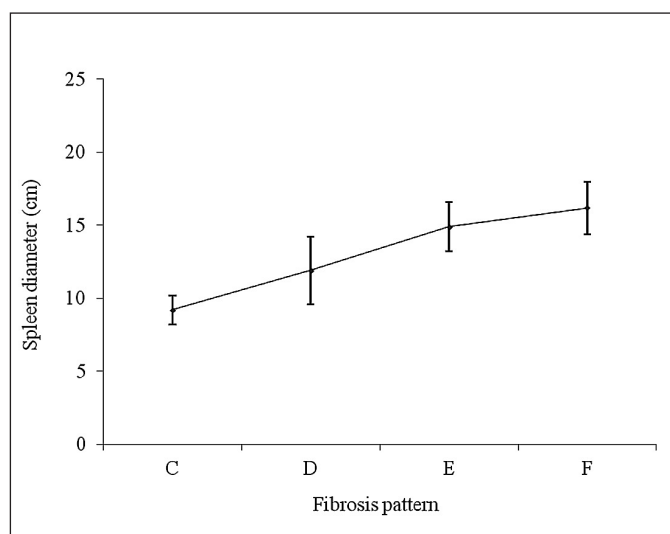


FIGURE 2. Distribution of mean spleen diameter (cm) according to periportal fibrosis pattern (Niamey classification) in 187 patients with *Schistosomiasis mansoni* (ANOVA $F = 108.53$; $P < 0.001$)

Figure 3 demonstrates a significant correlation between spleen diameter (in cm) determined using ultrasound and the mean platelets counts among the 187 patients with SM ($r = 0.758$; $P < 0.001$).

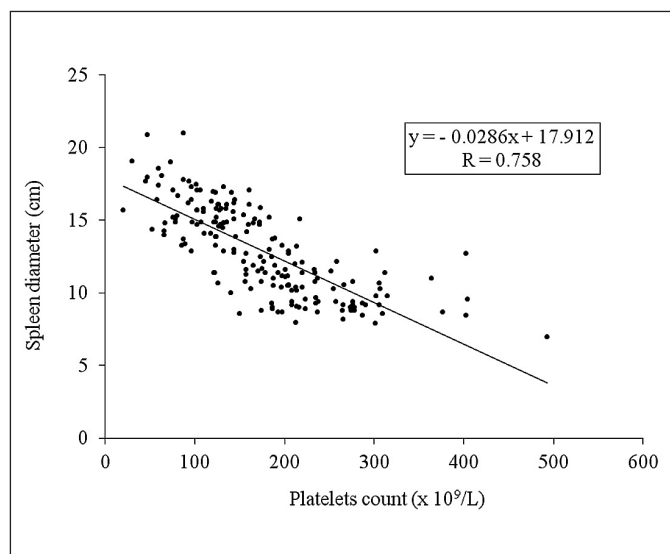


FIGURE 3. Correlation between spleen diameter (cm) and platelet count ($\times 10^9/L$) in 187 patients with *Schistosomiasis mansoni* ($P < 0.001$)

DISCUSSION

The present findings demonstrate that severe cases of SM continue to occur, as nearly half (45%) of the patients analyzed exhibited advanced patterns of periportal fibrosis (E and F). These patients with more advanced fibrosis patterns and with both platelet count and spleen size outside the range of normality have the hepatosplenic form of disease⁽⁸⁾. Additionally, they presented higher levels of portal pressure and a consequently greater risk of digestive bleeding^(6, 21).

In clinical practice, propaedeutic methods for the diagnosis, measurement and follow up of periportal fibrosis continue to be necessary. Therefore, a simple method as platelets count can be taken into the field for monitoring patient with portal hypertension in endemic areas.

The recent advent of hepatic elastography allows the measurement of the rigidity of the liver parenchyma and offers the same advantages as ultrasound scan in that it is a noninvasive procedure that allows evaluating a large portion of the parenchyma and can be employed repeatedly throughout cirrhotic patient follow up. The main advantages of elastography, however, include the determination of a numeric mean for some measurements and the fact that it is less dependent on the operator than an ultrasound exam, thereby allowing its use by healthcare professionals without the need for vast experience⁽⁹⁾. Nonetheless, the equipment is expensive and there are no data on its accuracy regarding the evaluation of periportal fibrosis in SM.

Due to the drawbacks of liver biopsies, blood markers involved in the metabolism of collagen have been investigated in periportal fibrosis^(10, 19, 27). Using ultrasound with the Niamey classification as the gold standard, correlations have been described between patterns of fibrosis and serum levels of IgG and hyaluronic acid^(5, 22). Though, the determination of these substances requires sophisticated methods that are generally not available in simpler laboratories found in endemic regions for schistosomiasis.

An inverse correlation has been described between periportal fibrosis and platelet count in patients with SM. Indeed, Koepke-Aguiar et al.⁽¹¹⁾ found more severe thrombocytopenia in patients with advanced forms of hepatosplenic schistosomiasis with portal hypertension. In addition, studying 47 patients with SM and 13 controls, Lambertucci et al.⁽¹²⁾ found a mean number of $194 \times 10^9/L$ platelets in the controls and $44 \times 10^9/L$ in patients with severe periportal fibrosis, as assessed by ultrasound. In this same study, the AST to Platelet Ratio Index (APRI) was also calculated to measure fibrosis, which demonstrated similar accuracy to that achieved with the platelet count.

In our study involving 187 patients, the mean platelet count was progressively lower with the increase of the fibrosis severity measured using ultrasound. It should be stressed that this is the first study to demonstrate a strong correlation between the platelet count and the four patterns of periportal fibrosis of the Niamey classification, as well as an inverse correlation between the mean platelet number and spleen diameter. Thrombocytopenia in patients with SM is believed

to stem from the retention of platelets in the parenchyma of the spleen^(5, 15, 23). In fact, in our study, thrombocytopenia was greater in those patients with a more enlarged spleen, denoting more advanced patterns of fibrosis (E + F).

The correlations found in the present study likely stem from the pathophysiology of portal hypertension in SM. There is an increased resistance to blood flow, as in cirrhosis, due to fibrosis secondary to the deposition of eggs in the portal branches, along with an increase in blood flow in the portal vein due to the splenomegaly induced by both the parasites and passive congestion^(18, 21, 24). Thus, a greater quantity of eggs (parasite load) may cause both a greater fibrotic reaction around the egg as well as greater difficulty in hepatic blood flow and greater splenomegaly due to immunological stimulation and congestion, which may lead to the greater sequestration of platelets⁽⁵⁾.

The strong correlation between the number of platelets and the spleen diameter observed in SM could also be strengthened by the absence of other factors that induces thrombocytopenia, which are found in the cirrhosis⁽²⁵⁾. For example, in this parasitic disease the production of thrombopoietin is higher than described in the cirrhotic patients, as well as in the SM antibodies antiplatelets are not observed as found in the HCV chronic liver disease^(11, 25).

Recently, a smaller number of platelets and larger spleen diameter have been described in patients with SM and with esophageal varices in comparison to those without varices⁽¹⁾.

These authors propose an index (platelets count/spleen diameter ratio) for the noninvasive diagnosis of esophageal varices in patients with SM⁽¹⁾.

According to the present findings, platelet count in patients with SM was inversely correlated with the severity of periportal fibrosis and spleen diameter. Therefore, the platelet count constitutes a tool for the evaluation of periportal fibrosis severity in patients with SM, offering the advantages of high accuracy, simplicity and low cost. However, further studies are needed to validate these data and compare the platelet count to other markers of periportal fibrosis in SM.

Authors' contributions

Tibério B Medeiros and Ana Lucia C Domingues conceive and design the study; acquisition of data; analysis and interpretation of data; Carlos F Luna interpretation of the data and statistical analysis; Edmundo P Lopes analysis and interpretation of data; drafting of the manuscript. All authors read and approved the final manuscript.

Ethical clearance

Prior to inclusion, the patients received information on the objectives and procedures and agreed to participate by signing a statement of informed consent. This study received approval from the Human Research Ethics Committee of the UFPE Center for Health Sciences.

Medeiros TB, Domingues ALC, Luna CF, Lopes EP. Correlação entre o número de plaquetas e o grau de fibrose hepática e o tamanho do baço em pacientes com *Esquistossomose mansoni*. Arq Gastroenterol. 2014,51(1):34-8.

RESUMO - Contexto - Estudos vem descrevendo correlação entre o número de plaquetas e o grau de fibrose hepática na hepatite viral crônica, mas poucas publicações estudaram esta correlação em pacientes com *Esquistossomose mansoni*. **Objetivo** - Correlacionar a contagem de plaquetas com o padrão de fibrose periportal e com o diâmetro do baço, avaliados pela ultrassonografia em pacientes com *Esquistossomose mansoni*. **Método** - Os pacientes com *Esquistossomose mansoni* foram avaliados pela ultrassonografia abdominal, por um único examinador, para determinação do padrão de fibrose periportal (classificação de Níamey) e do diâmetro do baço. A contagem de plaquetas foi realizada em contador automatizado. **Resultados** - Cento e oitenta e sete pacientes com *Esquistossomose mansoni* com média de idade de 50,2 anos foram incluídos no estudo, 114 (61%) dos quais eram mulheres. De acordo com a classificação de Níamey, a ultrassonografia revelou que 37, 64, 64 e 22 pacientes exibiam padrões C, D, E e F, respectivamente. Nestes quatro grupos, o número médio de plaquetas foi 264, 196, 127 e 103 x 10⁹/L, respectivamente, e o diâmetro médio do baço foi 9,2, 11,9, 14,9 e 16,2 centímetros, respectivamente. Observou-se, portanto, redução significativa na contagem de plaquetas associada à progressão da fibrose periportal e ao aumento do tamanho do baço. **Conclusão** - Neste estudo verificou-se que a contagem de plaquetas foi inversamente correlacionada com o padrão de fibrose periportal, como também com o diâmetro do baço nos pacientes com *Esquistossomose mansoni*.

DESCRITORES - Fibrose hepática. Contagem de plaquetas. Esplenomegalia. Trombocitopenia. Hipertensão portal. *Esquistossomose mansoni*.

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