The ARRIVE Guidelines Checklist

**Assessment of Periportal Fibrosis in *Schistosomiasis mansoni* patients by Proton nuclear magnetic resonance-based metabonomics models.**

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**ITEM**

**ITEM RECOMMENDATION Reported on page #**

01

**Assessment of Periportal Fibrosis in *Schistosomiasis mansoni* patients by Proton nuclear magnetic resonance-based metabonomics models.**



Title 1

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| Abstract 2  The evaluation of Periportal fibrosis (PPF) is essential for a prognostic assessment of patients with schistosomiasis mansoni. WHO Protocol Niamey defines patterns of fibrosis from abdominal ultrasonography, Nuclear Magnetic Resonance 1H NMR-based Metabonomics has been employed to assess liver fibrosis in some diseases. We aimed to build 1H NMR- based metabonomics models (MM) to discriminate mild from significant periportal PPF and identify differences in the metabolite profiles.A prospective cross-sectional study was performed on schistosomiasis patients at a University Hospital in Northeastern Brazil. We evaluated 41 spectra of serum samples from 10 patients with mild PPF (C Niamey pattern) and 31 patients with significant PPF (D/E/F Niamey patterns). MM were built using partial least squares-discriminant analysis (PLS-DA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) formalisms. PLS-DA and OPLS-DA resulted in discrimination between mild and significant PPF groups with R2 e Q2 values of 0.80 and 0.38 and 0.72 and 0.42 for each model, respectively. The OPLS-DA model presented accuracy, sensitivity and specificity values of 92.7%, 90.3% and 100% to discriminate significant PPF. The metabolites identified as responsible by discrimination were: N-acetylglucosamines, alanine, glycolaldehyde, carbohydrates and valine. The MMs were able to discriminate mild from significant PPF patterns in patients with schistosomiasis mansoni through identification of differences in serum metabolites profiles. |
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**INTRODUCTION**

Classification of the pattern of periportal fibrosis (PPF) is essential in the prognostic evaluation of patients with schistosomiasis mansoni.There is a need for novel methods minimally invasive and new biomarkers for the diagnosis schistosomiasis mansoni.

Background 3 3

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In the present study, we aimed to build 1H NMR-based MM to discriminate mild from significant PPF in patients with schistosomiasis mansoni and identify differences in the profiles of the endogenous metabolites.

Objectives 4

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**METHODS**

**INTRODUCTION**

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This study was approved by the UFPE Research Ethical Committee involving human subjects, Protocol Number 3.222.710

Ethical statement 5

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A prospective cross-sectional study was performed on schistosomiasis patients at a University Hospital in Northeastern Brazil. We evaluated 41 spectra of serum samples from 10 patients with mild PPF (C Niamey pattern) and 31 patients with significant PPF (D/E/F Niamey patterns). MM were built using partial least squares-discriminant analysis (PLS-DA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) formalisms.

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Study design 6

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Patients aged 18 years or over diagnosed with schistosomiasis mansoni were included from the Schistosomiasis Clinic of the Gastroenterology Service of the Hospital das Clínicas, Universidade Federal de Pernambuco (UFPE), between March and December 2019. Schistosomiasis diagnosis was based on the clinical history of contact with water sources in endemic areas, report of previous treatment with praziquantel, associated with finding of PPF by US scan. Exclusion clinical criteria were: presence of fatty liver disease, cirrhosis or hepatocellular carcinoma, portal vein thrombosis, HIV, hepatitis B or C virus coinfection, or history of drug-induced liver injury or alcohol.

All patients were submitted to US scan after overnight fasting of about 8 hours, by the same examiner. According to the Niamey-Belo Horizonte Protocol, PPF pattern was defined as follows: C (peripherical fibrosis), D (central fibrosis), E (advanced fibrosis) and F (very advanced fibrosis) patterns. Patients without or with a doubtful PPF (A and B pattern) were excluded of the study. All US exams were performed using a US Siemens Acuson S2000 instrument equipped with a 6C1 Ultrasound probe (Siemens Medical Solutions, Mountain View, CA, USA).

Blood samples were collected from a peripherical vein, after US scan. Serum was obtained after centrifugation (3500 rpm) using a Centurion-Laborline equipment. Liver function tests including alanine and aspartate aminotransferase (ALT and AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) and glycemia were carried out using Wiener Lab® kits in a Wiener Lab® autoanalyzer (Wiener Lab Group, Argentina). Part of the samples was stored at minus 40°C until the NMR analysis.

Experimental 7

05,06

procedures

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Experimental animals 8 **not applicable**

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Housing and husbandry 9 **not applicable**

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Sample size 10 The study was carried out by spontaneous demand involving human beings

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Allocating animals 11 **not applicable**

to experimental groups

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*Cross-sectional study—*Two metabonomics models were developed, as follows: PLS-DA metabonomics model presented accuracy equal to 0.85, R2 value equal to 0.80; OPLS-DA metabonomics model presented R2 and Q2 values equals to 0.717 and 0.417, respectively. We identified spectral regions more relevant for discrimination and we made assignments. This allowed to identified the endogenous metabolites associated to discrimination.

Experimental 12

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Outcomes

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Statiscal 13

To investigate the distribution of demographic and clinical or laboratory data between groups, univariate tests were performed using GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA) with unpaired Student's t-test, Mann-Whitney, and Fisher’s exact as appropriate. A *p*-value < 0.05 was set as the level of statistical significance. Multivariate statistical formalisms were employed, such as Principal Components Principal (PCA), Partial Least Square – Discriminant Analysis (PLS-DA) and Orthogonal Partial Least Square – Discriminant Analysis (OPLS-DA), using MetaboAnalyst online platform.

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Methods

**INTRODUCTION**

**RESULTS**

**RESULTS**

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Baseline data 14

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| Demographic and laboratorial characteristic of 41 patients with schistosomiasis mansoni | | | | |
| **Characteristic** | **Total** | **Mild PPF**  **(C pattern)** | **Significant PPF (D/E/F pattern)** | ***p*-value** |
| N | 41 | 10 | 31 | – |
| Age (years old) | 57 (18-80) | 48.1 (18–75) | 57.2 (25-80) | 0.0865a |
| Sex Male  Female | 17 (41%)  24 (59%) | 5 (40%)  5 (50%) | 12 (39%)  19 (61%) | 0.4820b |
| AST (U/L) | 29.0 ± 2.6 | 24.3 ± 1.9 | 30.0 ± 2.9 | 0.3328c |
| ALT (U/L) | 29.0 ± 2.2 | 26.2 ± 4.5 | 30.0 ± 3.2 | 0.4584c |
| ALP (U/L) | 262 ± 33 | 329 ± 113 | 238 ± 22 | 0.6379c |
| GGT (/LSN) | 62 ± 12 | 35 ± 16 | 71.2 ± 14.0 | **0.0013c** |
| Platelets count (/mm3) | 131 ± 12 | 218 ± 15 | 102 ± 11 | **0.0001c** |
| Total Cholesterol (mg/dL) | 169.0 ± 4.6 | 174.0 ± 7.2 | 167.8 ± 5.6 | 0.4626c |
| HDL (mg/dL) | 45.7 ± 2.0 | 49 ± 5.7 | 44.6 ± 2.0 | 0.4863c |
| LDL (mg/dL) | 105.0 ± 3.8 | 108 ± 4.7 | 104 ± 4.8 | 0.3760c |
| Glucose (mg/dL) | 93.6 ± 5.2 | 97 ± 14 | 92 ± 5.1 | 0.9451c |
| Data presented as Mean values ± standard deviation. a Unpaired *t* test; b Fisher’s exact test; c Mann-Whitney test. \* Mean value (lower limit – upper limit). – 10 patients with mild periportal fibrosis (PPF) and 31 with significant PPF, Pernambuco, Brazil, 2020. | | | | |

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Numbers analysed 15

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Forty-four patients were included, but three were excluded because their samples proved to be outliers. Thus, 41 patients with PPF were selected to the study, being 10 patients with C; 12 patients with D; 17 patients with E and two patients with F patterns, according to the Niamey-Belo Horizonte Protocol. These patients were divided into two group: mild PPF (C pattern) and significant PPF (D/E/F patterns).

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| Metabonomics models were validated by cross validation (LOOCV) and permutation test. However, we understanding that it is necessary more studies for external validation of metabonomics models built.  11 |

Outcomes and estimation 16

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Two supervised metabonomics models were built with 95% of confidence interval. The best resulted was obtained using OPLS-DA with 92.7% accuracy, 90.3% sensitivity, 100% specificity and PPV and NPV values equals to 100% and 76.9%, respectively.

Adverses events 17

**DISCUSSION**

In the present study, we used 1H NMR-based metabonomics from the serum of patients with *schistosomiasis mansoni* to discriminate among cases of more or less intensity according to the PPF pattern. Moreover, the metabonomics formalisms used enabled identification of some metabolites associated with the discrimination, such as: alanine, glycolaldehyde and N-acetylglucosamines which had higher serum levels in the significant PPF group; while valine and carbohydrates presented lower serum levels in the most severe cases.

These metabolic alterations were allowed to create metabonomics models based on NMR spectral data. Models built using the PLS-DA and OPLS-DA proved to be efficient to discriminate, in a minimally invasive and non-operator-dependent manner, patients with mild PPF from those with significant PPF.The metabolic signatures found are mainly related to the energy metabolism, liver function and control of tissue repair and architecture liver disease, in line with previous studies. These findings are fundamental in follow-up of these patients, mainly in endemic regions, where it is possible to collect a blood sample and dispense with the need for on-site devices, and examiners experienced. This will allow us to move forward in understand disease evolution mechanisms and be an alternative to conventional methods evaluation of PPF in patients with schistosomiasis.

were observed in relation to the sample size and the disproportionate among PPF standards. We intend to expand the study in the coming years in order to increase and balance the number of patients in each group.

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| 12  This technique can accurately detect even low-intensity infections, overcoming the limitations of current diagnostic techniques, with the use of a single serum sample. These models can be inserted in the propaedeutic arsenal in clinical practice for the measurement of PPF in remote áreas. |

Interpretation/scientific implications 18 Generabisability/ 19

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translation

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