

SHORT COMMUNICATION

Evaluation of Tests Based on the Antibody Response to Keyhole Limpet Haemocyanin and Soluble Egg Antigen to Differentiate Acute and Chronic Human Schistosomiasis Mansoni

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Specific IgG and IgM responses to soluble egg antigen (SEA) and keyhole limpet haemocyanin (KLH) were measured by ELISA in patients with acute and chronic schistosomiasis. The tests based upon IgM and IgG antibodies responses to KLH presented the best diagnostic discrimination, and can be used in conjunction with clinical and epidemiological data to the differential diagnosis of acute schistosomiasis.

Key words: keyhole limpet haemocyanin - soluble egg antigens - enzyme-linked immunosorbent assay

The diagnosis of schistosomiasis mansoni is classically made by stool parasitological techniques. In high and moderate prevalence areas, the detection of parasite eggs is simple and accurate (Tsang et al. 1983). However, the sensitivity of parasitological methods decreases in areas where the prevalence and intensity of transmission of schistosomiasis are low (Noya et al. 1997), and there are suggestions that, in this situation, serologic tests would be useful.

The discrimination of acute and chronic stages of the disease allows for early treatment and prevention of the severe forms of the disease. In addition, the possibility of diagnosing acute schistosomiasis contributes to defining geographic regions with active transmission (Valli et al. 1999). Although there are several serological approaches proposed to differentiate acute and chronic schistosomiasis, we have noticed some conflicting results (Verweij et al. 1995), justifying a re-evaluation of some of the proposed tests.

In this preliminary work we investigated the antibody responses to keyhole limpet haemocyanin (KLH) and soluble egg antigens (SEA) antigens, as markers of acute and chronic clinical forms of schistosomiasis.

We studied 54 patients with acute schistosomiasis from an unusual outbreak, caused by environmental imbalances

at Porto de Galinhas beach in 2000 (Barbosa et al. 2001) and 54 chronic patients including intestinal, hepatointestinal and hepatosplenic forms of schistosomiasis, from São Lourenço da Mata, an endemic area in the state of Pernambuco (Beck et al. 2001). The protocol study was approved by the Ethical Committee of the Centro de Pesquisas Aggeu Magalhães-Fiocruz.

Blood samples (5 ml) were taken with heparin (10 U/ml) and after centrifugation, the plasma was kept frozen at -20°C until testing. Specific IgG and IgM antibodies to SEA and KLH were measured by ELISA. For simplicity, we will denominate these tests KLH IgM, KLH IgG, SEA IgM, and SEA IgG. The general ELISA procedures were performed according to Yuesereng et al. (1994) for KLH antigen and Valli et al. (1997) for SEA antigen. We used KLH and SEA at concentrations of 2 ng/μl and 10 μg/ml, respectively, for the detection of IgM and IgG antibodies. The dilutions of plasma were 1:2000 for the KLH IgM and KLH IgG, and 1:400 and 1:200 for the SEA IgM and SEA IgG, respectively. The horseradish peroxidase goat anti-human Ig was 1000 fold diluted for the KLH tests, and 2500 and 3000 diluted, for the SEA IgM and SEA IgG, respectively. The SEA antigen was obtained according to Pearce et al. (1991); the protein content was determined by the method of Lowry et al. (1951), and aliquots were lyophilized in small aliquots before storing at -70°C.

The optical densities (OD) were transformed to AUE (arbitrary units of ELISA), that were defined as the ratio between the OD of the sample and the OD of a reference plasma. The cut-off was defined as the mean plus 1 standard error of plasma samples of chronic patients.

The groups with acute and chronic infection differed significantly respecting the levels of IgM and IgG antibodies to KLH ($p < 0.0001$ and $p < 0.0001$, respectively) and SEA ($p = 0.0002$ and $p < 0.0001$, respectively) (Figure).

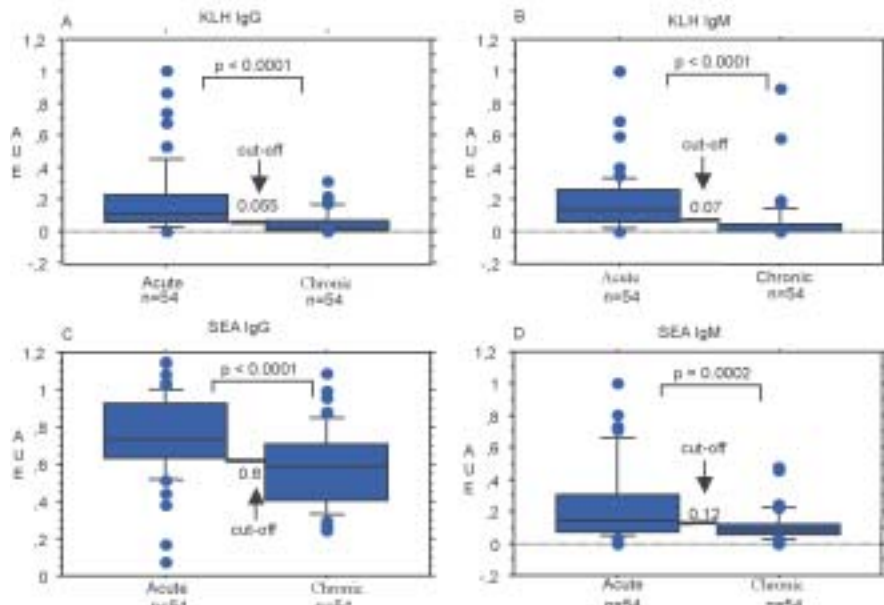
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Box plot percentiles of the levels of IgG and IgM antibodies to keyhole limpet haemocyanin (A and B), and soluble egg antigens (C and D), measured by ELISA in plasma of acute and chronic schistosomiasis patients, were expressed as AUE (arbitrary unit of ELISA). The cut-off is indicated by arrow.

Finally, we calculated the sensitivity to detect acute cases and the specificity to detect chronic cases for each assay. The sensitivities of KLH IgG, KLH IgM, SEA IgG and SEA IgM were 74.5, 70.9, 80 and 56.4%, respectively. The specificities of KLH IgG, KLH IgM, SEA IgG and SEA IgM were 72.2, 79.6, 51.9 and 75.9%, respectively. Thus, KLH IgM, and KLH IgG were the tests that displayed the best diagnostic discrimination, providing the best trade off between sensitivity and specificity were taken into account.

The results show that the evaluated assays can be used in conjunction with clinical and epidemiological data in attempts to discriminate acute and chronic schistosomiasis. Although we have focused in the discrimination of acute and chronic schistosomiasis in patients previously diagnosed with schistosomiasis, we are currently evaluating the possibility to use combinations of serological tests to simultaneously establish the diagnosis of schistosomiasis, and discriminate different clinical forms of the disease. In addition, we are analyzing more deeply the results using additional statistical tools, including receiver-operating characteristics analysis.

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