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The soluble worm antigens-specific antibodies used as biomarkers of *Schistosoma japonicum* in a low prevalence and intensity endemic area of Hubei, China

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Abstract

The precise diagnosis of *Schistosoma japonicum* (*S. japonicum*) infection plays a critical role in achieving the ultimate goal of eliminating schistosomiasis in endemic regions. We evaluated the *S. japonicum* soluble worm antigen protein (SWAP) specific-IgG, IgG₄ and IgE levels, and evaluated the association between *S. japonicum* infection and these antibodies in a sample of 837 residents from a *S. japonicum*-endemic area in Hubei province, China. The participants were divided into the Training Set (TS) and Validation Set (VS) based on the chronological order. Enzyme-linked immunosorbent assays were performed to detect the SWAP-specific antibodies. Three algorithms for identifying *S. japonicum* infection were generated in the TS and subsequently validated in the VS. The findings were further replicated in an independent cohort from an endemic area for *Schistosoma mansoni* (*S. mansoni*) in Brazil. Our results indicated for the first time that *S. japonicum*-infected individuals had higher levels of SWAP-specific IgG, IgG₄ and IgE, and lower value of the IgE/IgG₄ ratio than uninfected individuals in both the two Sets ($p < 0.01$). Both the infected and uninfected individuals had a high prevalence of seropositivity for IgG. We further showed that the predictive model EGR (IgE/IgG₄ ratio) score performed best in Chinese population (area under the receiver operating characteristic (AUROC) 0.905, sensitivity 82.7%, specificity 84.0% in the TS; AUROC 0.933, sensitivity 87.7%, specificity 89.1% in the

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Supplementary data associated with this article

VS). Nevertheless, the predictive model IgG₄ score performed best in Brazilian cohort (AUROC 0.788, sensitivity 73.2%, specificity 73.3%). In summary, SWAP-specific IgG could be used as a biomarker for identifying individuals who have been previously exposed to *S. japonicum*, and furthermore the SWAP-specific IgE/IgG₄ could be used as an immune biomarker for *S. japonicum* infection in particular in the endemic areas with low prevalence and intensity.

Keywords

Schistosomiasis japonica ; IgG; IgE; IgG₄; IgE/IgG₄ ratio; biomarker

1. Introduction

Schistosomiasis caused by infection with *schistosoma* is a neglected tropical disease (NTD). Globally, over 250 million people are infected with *schistosoma* and an estimated 800 million are at risk of infection (Hotez et al., 2014; Liu et al., 2012; Steinmann et al., 2006; Zhou et al., 2005). For more than a half century, use of praziquantel has been the major strategy of the national schistosomiasis control program in China, which led to a decrease in prevalence of *Schistosoma japonicum* (*S. japonicum*) infection from 11.6 million in the 1950s to 0.73 million by 2004, and an estimated 185,000 people by 2013 (Xu et al., 2011; Zhang et al., 2016; Zhou et al., 2004). By the end of 2016, the national mean *S. japonicum* infection rate is 0.02% and 0.0078% in humans and bovines, respectively (Zhang et al., 2017). However, transmission has re-emerged in the fields along the Yangzi River due to changes in ecological and socioeconomic factors, including severe flooding, climate change, a highly migratory population, and the large population density (Lin et al., 2008b; Utzinger et al., 2015; Zheng et al., 2002; Zhou et al., 2008).

Inspired by the London Declaration and the WHO Roadmap for NTDs in 2012, it is believed that control and elimination of schistosomiasis will benefit from a precise, accurate, and affordable diagnosis (Rollinson et al., 2013; Utzinger et al., 2015). Hubei Province located in the middle reaches of Yangtze River was a highly endemic region in China. After the implementation of national schistosomiasis control program, the prevalence was largely reduced and reached the criteria of “infection control” (e.g., human prevalence < 5%) (Hao et al., 2009; Li et al., 2005; Xu et al., 2011). Up to 2013, it was reported that the geometric mean eggs per gram of feces (epg/feces) in human infected with *S. japonicum* was 5.08 by Kato-Katz (KK) method (He et al., 2018). According to the WHO’s standard, the lower threshold for moderate infection of *Schistosoma mansoni* (*S. mansoni*) or *S. japonicum* refers to 99 epg/feces and 399 epg/feces for the heavy infection, respectively (Montresor et al., 1998). Thus, Hubei province is currently characterized as a chronic infected region but with a “low intensity”. The KK test was previously considered the “gold standard” method for diagnosis of *S. japonicum* in China through direct counting the number of eggs in a fecal smear (Katz and Chaia, 1968; Katz et al., 1970). However, the chronically infected patients generally have low egg counts and the KK has been reported insensitive leading to the false negative results (Lin et al., 2008a; Yu et al., 1998). On other hand, the immunoassay with high sensitivity and specificity is an alternative method to identify the *S. japonicum* infection. In particular, antigens or antibodies used in the assay can be

customized as the catching reagent and parasite infection can be detected by direct or indirect serologic tests (Gomes et al., 2014; Gray et al., 2011). Previous studies demonstrate the roles of worm-specific IgE antibody (spIgE) in clearance of the *schistosoma spp.* parasite and the level of spIgE in serum can be monitored as a biomarker for resistance to infection and reinfection (Dunne et al., 1992; Hagan et al., 1991; Walter et al., 2006). Furthermore, elevated spIgE titers against worm antigens in older individuals chronically infected with *S. japonicum*, *S. mansoni* or *Schistosoma haematobium* (*S. haematobium*) correlate to the resistance of reinfection following chemotherapy (Pinot de Moira et al., 2010; Rihet et al., 1991; Webster et al., 1997). In contrast, Worm-specific IgG₄ antibody (spIgG₄) antibody associates with susceptibility of infection and may even attenuate the protective effect of spIgE (Caldas et al., 2000; Hagan et al., 1991; Jiz et al., 2009). Together a low spIgE/spIgG₄ ratio is associated with a high rate of infection and reinfection of *S. mansoni* (Figueiredo et al., 2012; Pinot de Moira et al., 2010). Thus, the ratio between spIgE and spIgG₄ may help determine resistance or susceptibility to schistosomiasis. However, up to date, there is no study to clarify the association between adult worm antigen preparation (SWAP)-specific IgE and IgG₄ levels in *S. japonicum* infection. In this study, we assessed the prevalence of *S. japonicum* SWAP-specific IgG, IgG₄ and IgE in individuals who lived in the endemic area of Hubei province, and evaluated the humoral immune responses mediated by these SWAP-specific antibodies and whether these antibodies could be used as biomarkers of infection further studied.

2. Materials and methods

2.1. Study population

We enrolled 837 participants living in a *S. japonicum*-endemic area in Hubei province, of which 479 participants were infected with *S. japonicum*. The standard diagnostic procedure for *S. japonicum* infection in China includes KK examination and indirect hemagglutination assay (IHA) in combination with the frequency of water contact, clinic symptoms, the results of blood testing as well as abdominal ultrasound examination, respectively (Yu et al., 2007). To decrease the false-negative rate, if someone had negative KK examination and IHA but had frequent water contact, abnormal liver function or positive findings in ultrasonography, we would order the repeated feces examination through the government agents from the local disease control center to confirm either positivity or negativity of eggs after the enrichment of eggs in feces. In this way, the remaining 358 participants were diagnosed as exposed but without infection. According to the current regiment protocol, the participants diagnosed with *S. japonicum* infection will receive the anti-helminthic therapy as a 60 mg/kg praziquantel, divided into six doses and administered over 2 days (Deng et al., 2015). Serum samples were collected from participants prior to praziquantel therapy. Since the China's Schistosomiasis Control in 1992, we could assume that all the participants in the present study had accepted praziquantel treatment previously, whereas we ensured that all patients in current study have not used praziquantel for at least a year. Two 10 ml tubes were used, in which one had anticoagulant EDTA used for PCR and another one had coagulant used for serum separation. Generally, 3 to 5 ml serum could be separated from 10 ml blood and further aliquoted and stored at -80°C until antibody testing. Demographic data and medical history were collected as previously described (Long et al., 2017). 441

participants living in a *S. mansoni* endemic area in Conde, Brazil (as described previously) were enrolled to replicate the findings in *S. japonicum* in Hubei, China (Figueiredo et al., 2012).

The antibody study for *S. japonicum* was conducted in two phases including the Training set (TS) and the Validation set (VS) phase. Serum samples were processed in chronological order according to the date that the samples were collected. In addition, 15 healthy North American adults with no reported exposure to *S. japonicum* were included as secondary controls. Pregnant women and individuals using immunosuppressive drugs at the time of biospecimen collection were excluded. This study was approved by the Institutional Review Boards (IRBs) of Tongji Hospital, Huazhong University of Science and Technology (HUST), Johns Hopkins University School of Medicine, the University of Colorado School of Medicine and the Federal University of Bahia and was endorsed by the National Commission for Ethics in Human Research in Brazil.

2.2. Soluble *S. japonicum* worm antigenic protein (SWAP)

S. japonicum-infected rabbits were euthanized with CO₂ inhalation and the adult worms were collected by perfusion of the mesenteric vein. Adult worms were rinsed in saline and stored at -80°C (Figueiredo et al., 2012; Pellegrino and Siqueira, 1956). Equivalent numbers of male and female *S. japonicum* worms were quickly thawed in a 37°C water bath and homogenized on ice with a high salt buffer (400 mM KCl, 66.7 mM Tris and 66.7 mM EDTA, adjusting pH 8.0 with HCl) in a tissue grinder. Grinding was continued until no intact worms remained. The worm suspension was then centrifuged three times at 10,000 × *g* at 4°C for 3 minutes, followed each time by the addition of high salt buffer. The supernatants were combined, dialyzed against cold phosphate-buffered saline (PBS) (Gibco, Invitrogen, USA) at 4°C overnight and then sterile filtered (0.22 µm), aliquoted and stored at -80°C until used. A protein analysis (BioRad, Hercules, CA, USA, total protein kit) indicated that the extracted antigenic protein preparation contained 5.7 mg/ml of total protein.

2.3. SWAP-specific IgG removal from serum prior to SWAP-specific IgE analysis

Protein G-agarose (HiTrap Protein G HP, GE Healthcare Life Sciences, Product 17-0405-01) was applied to remove IgG prior to detecting SWAP-specific IgE (Souza-Atta et al., 1999).

2.4. SWAP-specific IgG, IgG₄ and IgE antibodies assays

The relative concentration of SWAP-specific IgG, IgG₄ and IgE in each serum was assessed by enzyme-linked immunosorbent assay (ELISA). Briefly, SWAP (1:1000 of the 5.7 mg/ml total protein stock) was coated on the 96 well plastic microtiter plate at 4°C overnight and then the plate was blocked with PBS buffer containing 1% bovine serum albumin (BSA, Sigma Chemical Co, St. Louis, MOUSA) for 1 hour at room temperature. Diluted test (infected and uninfected) serum and control serum were added in the plate (1:100 for IgG assay, 1:50 for IgG₄ assay, 1:4 for IgE assay) and incubated for 2 hours at room temperature, then biotin-conjugated monoclonal anti-human antibodies (IgG: clone HP6043B, IgG₄: clone HP6025B, IgE: clone HP6029B, 1 mg/ml, Hybridoma Reagent Laboratory, Baltimore, MD USA) and Avidin-horseradish peroxidase (HRP) (1 mg/ml; Sigma Chemical Company,

St. Louis, MO, USA) were added and incubated for 1 hour at room temperature, respectively. Finally, the substrate 2,2'-azino-dis-(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma Chemical Co, St. Louis MO USA Cat A1888, ABTS) was added and the stop solution (2 mM sodium azide) was used to stop the reaction. The optical density (OD) was measured using a microtiter plate reader at 450 nm.

A heterologous antibody (dose) vs optical density (response) reference curve was established by binding different amounts of IgG anti-SWAP in the reference serum. The reference serum was from an infected participant and was assigned 100 kU/ml. Serum from 15 healthy North American adults with no known exposure to *S. japonicum* were analyzed and $2 \times$ the mean semi-concentration was used to establish the positive/negative cutoff value.

2.5. Statistical Analysis

All data were analyzed using SPSS version 21.0 (SPSS, Chicago, IL, USA) and MedCalc software version 12.2.1.0 (MedCalc Software, Mariakerke, Belgium). Continuous variables were expressed as the mean $\pm$ standard deviation (SD) and categorical variables were expressed as the number and the percentage. Comparisons of continuous variables were performed using the Mann-Whitney U-test, and a Pearson chi-square analysis or Fisher's exact test was used to compare categorical variables, with no attempt to control for age or gender. Prior to analyzing IgE and IgG₄ levels, the kU/mL values were multiplied by a hundred and then log 10 transformed. A binary logistic regression analysis adjusted for age and gender was performed to generate algorithms for identifying *S. japonicum* infection in the TS (IgE score, IgG₄ score and EGR score) and subsequently validated in the VS. For replication of findings, three algorithms for identifying *S. mansoni* infection were also generated and evaluated in Brazilian cohort. The area under the receiver operating characteristic curve (AUROC) of each model was calculated to assess the discriminative ability of different models for identification of *S. japonicum*. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood (+LR), negative likelihood (-LR), proportion of well classified and their 95% confidence intervals (95% CI) were calculated. The De Long test was used to compare the AUROCs. The McNemar's test was used to compare sensitivity and specificity of the three models for identifying *S. japonicum* infection. $p < 0.05$ was considered to be significant difference.

3. Results

3.1. Baseline characteristics and *S. japonicum* SWAP-specific IgG, IgG₄ and IgE humoral immune responses

A total of 837 individuals who lived in *S. japonicum*-endemic area in Hubei province were included in the study. There were 98 (49.5%) infected and 100 (50.5%) uninfected participants in the TS, and 381 (59.6%) infected and 258 (40.4%) uninfected ones in the VS, respectively (Table 1). In general, infected individuals were older than uninfected individuals (56.8 ± 9.3 vs 41.1 ± 10.9 in the TS and 54.4 ± 8.3 vs 41.6 ± 7.0 in the VS). The interval between recruiting TS and VS was two years. All residents were given their unique identification number to ensure there was no overlap of recruitment occurred between the groups. More than half of the individuals were male (65.3% in the TS and

62.7% in the VS). Notably, the infected individuals showed higher values of SWAP-specific IgG, IgG₄ and IgE, and a higher prevalence of seropositivity for SWAP-specific IgG, IgG₄ and IgE than uninfected individuals in both the TS and VS ($p < 0.001$). Especially for SWAP-specific IgG, both the infected individuals and the uninfected ones had a high prevalence of seropositivity in the TS (96.9% vs 58.0%), as well as in the VS (99.2% vs 62.0%). The positive/negative cut-off threshold based on binding levels from 15 North American non-endemic controls was 0.20 kU/mL for IgG, 0.049 kU/mL for IgG₄ and 0.28 kU/mL for IgE. Uninfected individuals had a higher IgE/IgG₄ ratio than infected ones in both the TS (6.35 ± 4.13 vs 3.84 ± 5.48 , $p < 0.001$) and the VS (6.82 ± 3.04 vs 3.97 ± 6.69 , $p < 0.001$).

3.2. Models for identifying *S. japonicum* infection

We generated three algorithms to identify patients with *S. japonicum* in the TS using a binary logistic regression analysis adjusted by age and gender, as shown in below:

IgE score = $-13.415 + 0.145 \times \text{age} + 0.328 \times \text{gender (male: 1, female: 0)} + 4.830 \times \text{IgE}$;

IgG₄ score = $-8.788 + 0.151 \times \text{age} + 0.379 \times \text{gender} + 1.421 \times \text{IgG}_4$;

EGR score = $-5.909 + 0.149 \times \text{age} + 0.435 \times \text{gender} - 0.623 \times \text{IgE/IgG}_4 \text{ ratio}$.

We further assessed the discriminative ability of these three models in the TS. The AUROC and the parameters in identifying *S. japonicum* infection were as followed: IgE score: AUROC = 0.884 (95% CI, 0.839–0.928, $p < 0.001$), with sensitivity of 81.6%, specificity of 79.0%, PPV of 79.2%, NPV of 81.5%, +LR of 3.89, -LR of 0.23 and proportion of well classified 80.3%; IgG₄ score: AUROC = 0.903 (95% CI, 0.861–0.944, $p < 0.001$), with sensitivity of 92.9%, specificity of 75.0%, PPV of 78.4%, NPV of 91.5%, +LR of 3.71, -LR of 0.10 and proportion of well classified 83.8%; EGR score: AUROC = 0.905 (95% CI, 0.864–0.947, $p < 0.001$), with sensitivity of 82.7%, specificity of 84.0%, PPV of 83.5%, NPV of 83.2%, +LR of 5.17, -LR of 0.21 and proportion of well classified 83.3% (Figure 1A, Table 2). Although the IgG₄ score and EGR score had a better discriminating ability, there was no significant difference between the three models in De Long test (IgE score vs IgG₄ score $p = 0.153$; IgG₄ score vs EGR score $p = 0.327$; EGR score vs IgE score $p = 0.558$).

The Youden's index determined the ideal cutoff values for the three models as 0.454 for IgE score, 0.327 for IgG₄ score and 0.558 for EGR score, respectively (Figure 1A and Table 2). The IgE score correctly classified 159 of 198 individuals (80.3%, 80 true positive and 79 true negative) and misclassified 39 of 198 individuals (19.7%, 18 false-negative and 21 false-positive). The IgG₄ score correctly classified 166 of 198 individuals (83.8%, 91 true positive and 75 true negative) and misclassified 32 of 198 individuals (16.2%, 7 false-negative and 25 false-positive). The EGR score correctly classified 165 of 198 individuals (83.3%, 81 true positive and 84 true negative) and misclassified 33 of 198 individuals (16.7%, 17 false-negative and 16 false-positive). IgG₄ score had the best sensitivity and EGR the best specificity using McNemar's test. Comprehensively considering the sensitivity and

specificity, the EGR score with both sensitivity and specificity higher than 80.0% has the best identification capability of *S. japonicum* among the three models (Table 2).

3.3. Validating the models for identifying *S. japonicum* infection in the VS

We further applied these three models to calculate the scores of participants in VS and used their cutoff values as criteria to identify *S. japonicum* infection. Accordingly, the AUROC of IgE score, IgG₄ score and EGR score were 0.830 (95% CI 0.798–0.862, $p < 0.001$), 0.910 (95% CI 0.886–0.933, $p < 0.001$) and 0.933 (95% CI 0.912–0.954, $p < 0.001$), respectively (Figure 1B and Table 2). The EGR score had a significantly better discriminative ability than the two others (De Long test: EGR score *vs* IgE score $p < 0.001$, EGR score *vs* IgG₄ score $p = 0.005$). In terms of best sensitive and specificity, the EGR score also performed best among the three models using McNemar's test (IgE score of sensitivity 72.2% and specificity 83.7%; IgG₄ score of sensitivity 86.6% and specificity 84.5%; as well as EGR score of sensitivity 87.7% and specificity 89.1%, respectively) (Table 2).

3.4. Replicating the models in Brazilian cohort for identifying *S. mansoni* infection

Three algorithms to identify patients with *S. mansoni* were generated, as shown below:

$$\text{IgE score} = -1.5 - 0.019 \times \text{age} + 0.469 \times \text{gender (male: 1, female: 0)} + 0.7 \times \text{IgE};$$

$$\text{IgG}_4 \text{ score} = -2.844 - 0.039 \times \text{age} + 0.198 \times \text{gender} + 1.752 \times \text{IgG}_4;$$

$$\text{EGR score} = 0.314 - 0.022 \times \text{age} + 0.43 \times \text{gender} - 0.265 \times \text{IgE/IgG}_4 \text{ ratio}.$$

Although the IgG₄ score with AUROC 0.797, sensitivity 80.5% and specificity 55.2% had the best discriminating ability among the three models (De Long test: IgE score *vs* IgG₄ score $p < 0.001$; IgG₄ score *vs* EGR score $p < 0.001$; EGR score *vs* IgE score $p < 0.001$), the results were less superior compared to the one performed in *S. japonicum* infected Chinese cohort (Figure 1C and Table 3).

4. Discussion

A major challenge in schistosomiasis diagnosis is the lack of a precise, accurate, and affordable “gold-standard” test (Wilson, 2017). The KK test has been used for decades in the diagnosis of *S. japonicum* expect that the sensitivity ranges widely from 20% - 70% particularly in regions with lower (chronic) worm burdens following widespread treatment with Praziquantel, (Kongs et al., 2001; Lin et al., 2008b). Issue has been raised that the day-to-day variation of egg excretion still likely misses lightly or chronically infected individuals (Gray et al., 2011).

Immunodiagnostic techniques through detecting *schistosome* antigens or specific antibodies are more promising than parasitological diagnosis for their higher sensitivity and less time consuming, and may serves as the alternative approaches to identify *schistosomiasis* in low prevalence and intensity endemic areas (Wu, 2002; Zhu, 2005). (Doenhoff et al., 2004; Enk et al., 2008). The IgE/IgG₄ balance has been proved to play a role in resistance or susceptibility to infection (Figueiredo et al., 2012). The approach of measuring anti-adult

worm IgE, IgG₄ and the IgE/IgG₄ ratio was well established to identify infection status with *S. mansoni* (Figueiredo et al., 2012; Hagan et al., 1991; Jiz et al., 2009; Matoso et al., 2013). In the current study, we purposely studied the *S. japonicum* SWAP-specific IgG, IgG₄ and IgE antibodies in the cohort from Hubei characterized by low prevalence and intensity of *S. japonicum* infection. We found that all individuals who had exposure to *S. japonicum* developed the high seropositivity for SWAP-specific IgG regardless of their infectious status. Similarly, Xie and colleagues demonstrated that the *S. japonicum* SWAP-specific antibodies between individuals lived in endemic and non-endemic areas are comparable whereas the level of SWAP-specific IgG was higher in endemic area, though they did not observe the difference in levels of SWAP-specific IgG₄ and IgE between the two areas (Xie et al., 2012). Together, we suggest that SWAP-specific IgG could be used as a biomarker for identifying previous exposure to *S. japonicum*. We next generated three models based on those SWAP-specific antibodies and the IgE/IgG₄ ratio to assess their discriminative ability of *S. japonicum* infection in two different sets in Chinese cohort and further replicated the models in *S. mansoni* infected Brazilian cohort. Given to that the levels of *schistosome*-specific antibodies in uninfected individuals are different between endemic and non-endemic areas, we enrolled individuals exposed but uninfected as controls to avoid being misdiagnosed with infection in the endemic areas. Age and gender associated with antibodies response against *schistosome* were taken into the models as covariates (Naus et al., 2003; Webster et al., 1997). In the TS, three models failed to show significant difference in identifying the *S. japonicum* infection. However, the EGR score performed superior showing higher than 80.0% for both sensitivity and specificity. In the VS, we scored all the participants with the models generated in the TS and found that the EGR score had the best discriminative ability. To our knowledge, we present the first time that SWAP-specific IgE, IgG₄ and the IgE/IgG₄ ratio can serve as predictive biomarkers to identify *S. japonicum* infection. When Lin and colleagues used anti-egg antigen IgG-ELISA kits for diagnosis of *S. japonicum* in low intensity endemic area of China, they reported poor performance of the kits (sensitivity of 79.3% - 87.4% and specificity of 38.9% - 53.5%) and observed a trend of the reduced sensitivity towards lower intensity infection (Lin et al., 2008b). In Brazilian cohort, we found that IgE, IgG₄ and IgE/IgG₄ ratio were all associated with *S. mansoni* infection. Although the IgG₄ score performed best among three models, its discriminative ability was less effective than the EGR score used for *S. japonicum* in Chinese cohort. Accordingly, Matoso and colleagues found that the *S. mansoni* egg-specific IgG₄ had a higher predictive power than the ratio of IgG₄/IgE, but a lower predictive power of IgG₄ (AUROC: 66% - 84%) (Matoso et al., 2013). It remains unclear the reason of discrepancy appeared between *S. japonicum* and *S. mansoni*. By studying discriminative ability of SWAP-specific antibodies, we assume that the difference of pathogenesis, epidemic situation and infected status, all may play certain roles. The different pathogenesis between the *S. japonicum* and *S. mansoni* includes the cellular composition of granuloma and the related action of chemokines and immune inflammatory response, all of which may attribute to the disease severity and fibrotic process in liver (Chuah et al., 2014). In addition, our previous study reveals the disease progress and prognosis may be further linked to the difference of gene polymorphism from the infected hosts and their inflammatory responses in two types of schistosomiasis (Long et al., 2017). It is notable that geographical distribution is relatively diverse between the strains; for example, *S. mansoni* is endemic in sub-Saharan

Africa, Arabian Peninsula, Middle East, and South America, particularly in Brazil, while *S. japonicum* is endemic in China, Philippines, and Indonesia (Chuah et al., 2018). Studies have reported that infection intensity correlates with the antibody profile (Gaze et al., 2014; Xu et al., 2014). In comparison of infection intensity, Hubei province has the *S. japonicum* infection rate of 0.09% by the end of 2016 (Zhu et al., 2018). In contrast, the Brazilian individuals enrolled in the present study have a relative high infection rate of *S. mansoni* (37.7%) and 27.9% of the infected individuals have moderate-heavy intensity infection (Figueiredo et al., 2012). Given to that the excellent predictive power of immunodiagnostic techniques have been reported (Cai et al., 2017; Xu et al., 2011; Xu et al., 2014), the present study is another preliminary exploration using *schistosome*-specific IgE, IgG₄ antibodies as biomarkers for diagnosis.

There are some limitations in the current studies. For example, we used the SWAP antigens which were crude extracts consisting of thousands of *schistosome* proteins that might be shared with unrelated pathogens and therefore may impair the accuracy (Grenfell et al., 2013; Xu et al., 2014). In addition, when studying Hubei cohort, other trematodes' infection history was absent, of which may shy away from the antibody cross-reaction issues. Boamah and colleagues use immunoproteomics to determine the major IgE and IgG₄ responding to *S. japonicum* adult worm antigens (Boamah et al., 2012). In the future study, similar methods will be used in parallel to find an optimal way of improving the predictive power.

Secondly, KK test may not be accurate for those mildly or chronically infected cohort. Furthermore, it is well known that antibody-based assays may fail to distinguish between the previously and actively infected patients (Wu et al., 2018). Nevertheless, the Chinese cohort we studied is from the endemic areas, in which all residents have unique ID number and the local disease control center conducts survey each year to record the newly infected cases and monitor the previous infection. Inquiry of the past medical records, recent water contact history and clinical symptoms may help to distinguish the recently infected cases. In addition, for those past infected cases, the frequency of water contact, clinic symptoms, the results of blood testing such as liver function panel as well as abdominal ultrasound examination are also taken into consideration (Yu et al., 2007). Given to that some studies have reported that the recombinant antigens might have the ability to distinguish the active infection from the past infection (Hinz et al., 2017; Jin et al., 2010; Xu et al., 2014), and the subject will be warranted for the further investigation. Finally, serum samples in both the TS and VS came from the same endemic area and in future, this approach need to verified in other regions.

In conclusion, our data suggests that the SWAP-specific IgG could be used as a biomarker for identifying individuals who have been previously exposed to *S. japonicum*, while the SWAP-specific IgE/IgG₄ could be used as an immune biomarker for identifying *S. japonicum* infection in low prevalence and intensity endemic areas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Receiver operating characteristics curves (ROC) analysis for identifying *S. japonicum* infection and *S. mansoni* infection. A, In the Training Set ($n = 198$), there was no significant difference of discriminative ability among the three models (De Long test: IgE score vs IgG₄ score $p = 0.153$; IgG₄ score vs EGR score $p = 0.327$; EGR score vs IgE score $p = 0.558$); B, in the Validation Set ($n = 639$), the EGR score performed best (De Long test: IgE score vs IgG₄ score $p < 0.001$; IgG₄ score vs EGR score $p < 0.001$; EGR score vs IgG₄ score $p = 0.005$); C, and in Brazilian cohort ($n = 441$), the IgG₄ score had the best discriminative ability (De Long test: IgE score vs IgG₄ score $p < 0.001$; IgG₄ score vs EGR score $p < 0.001$; EGR score vs IgE score $p < 0.001$).

Table 1Baseline characteristics and SWAP-specific IgG, IgG₄ and IgE humoral immune responses in China populaion

Variables	TS (n=198)	TS with infection (n=98)	TS without infection (n=100)	p value	VS (n=639)	VS with infection (n=381)	VS without infection (n=258)	p value	VS vs TS p value
Age (year)	48.9 ± 12.8	56.8±9.3	41.1 ± 10.9	<0.001	49.1 ± 10.1	54.4 ± 8.3	41.6 ± 7.0	<0.001	0.802
Gender (male)	108 (54.5%)	64 (65.3%)	44 (44.0%)	0.003	348 (54.5%)	239 (62.7%)	109 (42.2%)	<0.001	0.983
IgG (kU/mL)	1.82 ± 2.96	3.18 ± 3.70	0.49 ± 0.59	<0.001	1.87 ± 2.56	3.11 ± 3.51	0.51 ± 0.54	<0.001	0.817
IgG (positive)	153 (77.3%)	95 (96.9%)	58 (58.0%)	<0.001	538 (84.2%)	378 (99.2%)	160 (62.0%)	<0.001	0.025
IgE (kU/mL)	0.23 ± 0.23	0.29 ± 0.31	0.17 ± 0.04	<0.001	0.21 ± 0.15	0.24 ± 0.18	0.17 ± 0.09	<0.001	0.078
IgE (positive)	22 (11.1%)	20 (20.4%)	2 (2.0%)	<0.001	91 (14.2%)	78 (20.5%)	13 (5.0%)	<0.001	0.260
IgG ₄ (kU/mL)	0.74 ± 2.24	1.36 ± 3.03	0.14 ± 0.54	<0.001	0.88 ± 2.87	1.44 ± 3.61	0.06 ± 0.09	<0.001	0.531
IgG ₄ (positive)	82 (41.4%)	65 (66.3%)	17 (17.0%)	<0.001	287 (44.9%)	238 (62.5%)	49 (19.0%)	<0.001	0.386
IgE/IgG ₄ ratio	5.41 ± 5.36	3.97 ± 6.69	6.82 ± 3.04	<0.001	4.85 ± 5.13	3.84 ± 5.48	6.35 ± 4.13	<0.001	0.188

TS, Training Set; VS, Validation Set

Table 2

Performance of the three models for identifying *S. japonicum* infection in the Training Set and Validation Set

	AUROC (95% CI), <i>p</i>	<i>p</i> ^a	Cutoff value	Sensitivity (95% CI), <i>p</i> ^b Specificity (95% CI), <i>p</i> ^b	PPV, % NPV, %	+LR -LR	Well classified
IgE score							
TS	0.884 (0.839–0.928), <i>p</i> < 0.001	0.153	0.454	81.6 (72.3–88.5), <0.001	79.2 (69.8–86.4)	3.89 (2.63–5.75)	80.3%
VS	0.830 (0.798–0.862), <i>p</i> < 0.001	< 0.001		79.0 (69.5–84.2), 0.344	81.5 (72.0–88.3)	0.23 (0.15–0.35)	
				72.2 (67.3–76.6), <0.001	86.8 (82.4–90.2)	4.43 (3.34–5.89)	76.8%
				83.7 (78.5–87.9), 0.880	67.1 (61.6–72.1)	0.33 (0.28–0.39)	
IgG ₄ score							
TS	0.903 (0.861–0.944), <i>p</i> < 0.001	0.647	0.327	92.9 (85.3–96.8), 0.002	78.4 (69.7–85.3)	3.71 (2.63–5.24)	83.8%
VS	0.910 (0.886–0.933), <i>p</i> < 0.001	0.005		75.0 (65.2–82.9), 0.004	91.5 (82.7–96.2)	0.10 (0.05–0.20)	
				86.6 (82.7–89.8), 0.652	89.2 (85.5–92.1)	5.59 (4.19–7.45)	85.8%
				84.5 (79.4–88.6), <0.001	81.0 (75.7–85.4)	0.16 (0.12–0.20)	
EGR score							
TS	0.905 (0.864–0.947), <i>p</i> < 0.001	0.138	0.558	82.7 (73.4–89.3), 1.000	83.5 (74.3–90.0)	5.17 (3.27–8.17)	83.3%
VS	0.933 (0.912–0.954), <i>p</i> < 0.001	< 0.001		84.0 (75.0–90.3), 0.227	83.2 (74.1–89.6)	0.21 (0.13–0.32)	
				87.7 (83.8–90.7), <0.001	92.3 (88.9–94.7)	8.08 (5.68–11.48)	88.3%
				89.1 (84.5–92.5), 0.044	83.0 (78.0–87.2)	0.14 (0.11–0.18)	

TS, Training Set; VS, Validation Set; AUROC, area under receiver operating characteristic; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood; -LR, negative likelihood.

^aDe Long test;^bMcNemar-Bowker test.

Table 3
Performance of the three models for identifying *S. mansoni* infection in Brazil population

	AUROC (95% CI) <i>p</i>	<i>p</i> ^a	Cutoff value	Sensitivity (95% CI), <i>p</i> ^b Specificity (95% CI), <i>p</i> ^b	PPV, % NPV, %	+LR -LR	Well classified
IgE score	0.628 (0.576–0.680), <i>p</i> < 0.001	<i>p</i> < 0.001	0.783	82.9 (76.1–88.2) 40.8 (35.0–45.9)	45.3 (39.6–51.2) 80.1 (72.4–86.2)	1.40 (1.24–1.58) 0.42 (0.30–0.59)	56.5%
IgG ₄ score	0.788 (0.745–0.831), <i>p</i> < 0.001	<i>p</i> < 0.001	0.395	73.2 (65.6–79.6) 73.3 (67.6–78.3)	61.9 (54.6–68.6) 82.2 (76.7–86.6)	2.74 (2.21–3.40) 0.37 (0.28–0.47)	73.2%
EGR score	0.693 (0.644–0.743), <i>p</i> < 0.001	<i>p</i> < 0.001	0.368	64.0 (56.1–71.3) 69.3 (63.5–74.6)	55.3 (47.9–62.4) 76.5 (70.7–81.5)	2.09 (1.69–2.58) 0.52 (0.42–0.64)	67.3%

AUROC, area under receiver operating characteristic; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood; -LR, negative likelihood.

^a, De Long test;

^b, McNemar-Bowker test.