Characteristics of Peripheral Lymphocyte Subsets in Patients with Different Stages of Schistosomiasis Japonica

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Abstract

Background Immune cells are important for the development of schistosomiasis japonica and are also critical for the treatment of schistosomiasis. The immune cells in the peripheral blood help assess the immune state. The peripheral lymphocytes in schistosomiasis mansoni were well studied, however immune cells in patients with different stages of Schistosomiasis Japonica are not well analyzed. Here we performed a preliminary study to explore characteristics of peripheral lymphocyte subsets in patients with different stages of Schistosomiasis Japonica. **Methods** 135 patients with S. japonicum infection and 25 healthy volunteers were included in this study, including 84 patients with chronic S. japonicum infection and 51 patients with advanced S. japonicum infection. Flow cytometry analysis was performed to evaluate peripheral lymphocytes including T cells, B cells and NK cells. Blood routine and liver function test data were analyzed. Ultrasound examination was used to access liver fibrosis according to the World Health Organization standard about ultrasound in schistosomiasis. **Results** Demographic data analysis suggested there was no difference in age and gender in patients with S. japonicum infection and health control group. Liver function tests showed that patients with advanced schistosomiasis had a higher incidence of liver function abnormality and blood lipid than those with chronic schistosomiasis. Blood routine results reflected that hemoglobin, red blood cells, platelets, as well as lymphocytes in the advanced group were significantly less than that in the chronic group. Furthermore, flow cytometry analysis indicated that the percentage of CD4 $^+$T cells was lower in the advanced group, but the percentage of CD19 $^+$B cells was higher in the advanced group. In addition, the number of CD3 $^+$T cells, CD3 $^+$CD4 $^+$T cells, CD3 $^+$CD8 $^+$T cells, and NK cells was less in the advanced group when compared with those in the chronic group. In addition, there was a correlation between the decrease in CD4 $^+$ T cells and more severe fibrosis on ultrasound images. **Conclusion** Our results indicated that the immune state in the peripheral is different in different stages of S. japonicum infection. Lymphocyte subset analysis have potential to facilitate differential diagnosis of different stages of schistosomiasis japonica and even to be a prognostic factor.

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Methods

135 patients with S. japonicum infection and 25 healthy volunteers were included in this study, including 84 patients with chronic S. japonicum infection and 51 patients with advanced S. japonicum infection. Flow cytometry analysis was performed to evaluate peripheral lymphocytes including T cells, B cells and NK cells. Blood routine and liver function test data were analyzed. Ultrasound examination was used to access liver fibrosis according to the World Health Organization standard about ultrasound in schistosomiasis.

Results

Demographic data analysis suggested there was no difference in age and gender in patients with S. japonicum infection and health control group. Liver function tests showed that patients with advanced schistosomiasis had a higher incidence of liver function abnormality and blood lipid than those with chronic schistosomiasis. Blood routine results reflected that hemoglobin, red blood cells, platelets, as well as lymphocytes in the advanced group were significantly less than that in the chronic group. Furthermore, flow cytometry analysis indicated that the percentage of $CD4^+$T cells was lower in the advanced group, but the percentage of $CD19^+$B cells was higher in the advanced group. In addition, the number of $CD3^+$T cells, $CD3^+CD4^+$T cells, $CD3^+CD8^+$T cells, and NK cells was less in the advanced group when compared with those in the chronic group. In addition, there was a correlation between the decrease in $CD4^+$ T cells and more severe fibrosis on ultrasound images.

Conclusion

Our results indicated that the immune state in the peripheral is different in different stages of S. japonicum infection. Lymphocyte subset analysis have potential to facilitate differential diagnosis of different stages of schistosomiasis japonica and even to be a prognostic factor.

Keywords: schistosomiasis, peripheral lymphocyte, Schistosoma japonicum, chronic schistosomiasis, advanced schistosomiasis, ultrasound examination

Introduction

Schistosomiasis is one of the most important parasite diseases, affecting over 200 million people in the world[1, 2,3,4]. It is caused by Schistosoma infection and the main disease-causing species are Schistosoma japonicum, Schistosoma mansoni, and Schistosoma haematobium. Schistosomiasis is the endemic in 78 countries in Africa, South America, and Asia, while Schistosomiasis japonica is mainly prevalent in Asia, particularly in China[5]. Although China has achieved tremendous successes in schistosomiasis control, China is still among the countries with the highest social and economic burden from schistosomiasis[6,7,8].

According to the disease progression, schistosomiasis japonica is classically divided into three distinctive clinical stages: acute schistosomiasis, chronic schistosomiasis, and advanced schistosomiasis. Acute schistosomiasis occurs when people contact with water containing cercariae. Patients with acute schistosomiasis will typically have a fever, diarrhea, abdominal pain, fatigue, and malaise[3,9,10,11]. Chronic schistosomiasis japonica develops after years of infection, which is caused by immunopathological reactions and chronic inflammation in reaction to eggs produced by mature schistosomes. Advanced schistosomiasis is the most severe stage of schistosomiasis japonica, associated with poor survival and prognosis. Patients with advanced schistosomiasis are usually accompanied by liver fibrosis, portal hypertension, ascites, splenomegaly, and gastro-esophageal variceal bleeding, or with a granulomatous disease of the colon [1, 12, 13]. As China is moving toward schistosomiasis elimination, very few acute schistosomiasis cases occur, however, there is still a large number of patients with chronic schistosomiasis or advanced schistosomiasis, representing a severe public health problem [6, 7]. Furthermore, the underlying mechanism remains not well defined and there is a lack of effective treatment. Thus, further study is needed to deepen the understanding of the pathological mechanism and promote the elimination of schistosomiasis.

Schistosomiasis is associated with multiple mechanisms, among which immune cells play a critical role in the development of schistosomiasis[14, 15]. The immunological status varies in different phases of schistosomiasis. Studies have demonstrated that both Th1 and Th2 immune responses get involved in the pathogenesis of schistosomiasis [16, 17]. In the initial phase of Schistosoma japonica infection, the immune responses are characterized by increased $CD4^+$IFN-γ^+T cells, TNF-α, and IL-6[18, 19]. However, in the chronic schistosomiasis stage, the immune responses shift from Th1 to Th2/regulatory phenotype, featured by higher expression of Th2 associated cytokines, such as IL-4, IL-5, IL-13, and IL-10[20]. Shreds of evidence also suggested that $CD4^+CD25^+Foxp3^+$Treg cells are increased in the stage of chronic schistosomiasis. In addition, higher expression of IL-17 was observed in this stage[18, 21]. Interestingly, lymphocytes from patients with advanced schistosomiasis didn't show a higher production of Th2 cytokines or proinflammatory cytokines when compared with patients in other stages, which is associated with immune exhaustion[22]. T cells are not only critical in the pathogenesis of schistosomiasis, but they are also important for the resistance to infection. Studies showed that peripheral T cells declined when infected with Schistosoma, however deletion of T cells abolished the resistance to Schistosoma infection[18]. In addition to T cells, B cells are also involved in the development of schistosomiasis. The expansion of Breg cells in the peripheral blood is regarded as an important feature in patients with chronic schistosomiasis[23, 24] As classic innate immune cells, NK cells also play a significant role in schistosomiasis[22, 25-27]. NK cells can negatively regulate Schistosoma japonica infection-induced liver fibrosis, as activated NK cells attenuated Schistosoma-induced liver fibrosis while deletion of NK cells dramatically enhanced liver fibrosis[27].

Immune cells are important for the development of schistosomiasis japonica and are also critical for the treatment of schistosomiasis. The immune cells in the peripheral blood help assess the immune state. The peripheral lymphocytes in schistosomiasis mansoni were well studied, however immune cells in peripheral blood in patients with different stages of Schistosomiasis Japonica are not well analyzed. In this study, we analyzed T cells, B cells, and NK cells in peripheral blood in patients with schistosomiasis, using flow cytometry. We compared these profiles among healthy volunteers and patients in different stages of schistosomiasis japonica to assess the frequency and number of those immune cells in patients with schistosomiasis. In addition to this, we selected patients with well-documented clinical data and analyzed the ultrasound images of the patients in an attempt to find the relationship between peripheral lymphocytes and imaging of the patients.

MATERIALS AND METHODS

Study Population

From March 2020 to April 2021, 135 patients from Changde, including 84 chronic and 51 advanced ones were included and 24 healthy volunteers were included as the control group. Changde, a city near Dongting Lake, has long been the epicenter of Schistosoma japonica outbreaks. Changde was the first place in China where Schistosoma japonicum was found, and even after considerable treatment by the government, a large number of Schistosoma patients still exist. Informed consent was obtained for each patient, and the study was approved by the Institutional Review Board of Third Xiangya Hospital, Central South University (No. 21149).

Patient Selection

According to the national standardized diagnostic criteria for schistosomiasi[28], the inclusion criteria for advanced schistosomiasis cases were: (1) history of repeated or prolonged exposure to infested water, or history of schistosomiasis treatment; (2) eggs found in the fecal examination, or positive serum immunology (enzyme-linked immunosorbent assay, ELISA); (3) portal hypertension syndrome resulting from hepatic fibrosis, splenomegaly [?] Hackett grade 3, or splenomegaly of Hackett grade 2 with hypersplenism or upper gastrointestinal bleeding or varices of esophagus and fundus of the stomach; and (4) portal hypertension syndrome resulting from hepatic fibrosis or splenomegaly or ascites, which were caused by schistosomiasis. The inclusion criteria for chronic schistosomiasis cases were: (1) history of repeated or prolonged exposure to infested water, or history of schistosomiasis treatment; (2) eggs found in the fecal examination, or positive

serum immunology (enzyme-linked immunosorbent assay, ELISA); (3) no obvious clinical symptoms, or intermittent abdominal pain, diarrhea or purulent blood stools, most were associated with hepatomegaly, mainly in the left lobe, and a few with splenomegaly. Exclusion criteria were pregnancy or diseases affecting the immune response (HIV, HTLV-1, and diabetes), individuals with other liver diseases associated with portal hypertension (e.g., hepatitis A, B, and C), and acute viral or bacterial infections at the time of blood collection, and patients who had not completed an immunologic evaluation.

Blood sample collection and processing

Blood samples were taken at random from proven Schistosomiasis patients who visited the Hanshou 3rd People's Hospital for treatment. Complete blood count was done using Mindray Bc5180 automated hematology analyzer (Mindray Industrial Co Ltd, P.R.China). And the liver function tests such as aspartate and alanine aminotransferases (AST and ALT), total bilirubin (TBIL), direct bilirubin (DBIL), albumin protein (ALB), total cholesterol (TC), Triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured by Mindray Bs800 fully automated biochemistry analyzer (Mindray Industrial Co Ltd, P.R.China).

Flow Cytometry

Within 3 hours after collection, blood samples were taken in heparinized tubes and delivered to the laboratory on ice. In a BD Trucount Absolute Count Tube, add 50 μl of blood, and 20 μl of BD Multitest 6-Color TBNK Reagent, mix well and set aside for 15 minutes. Add 450 μl of Lysate Red, shake well and protect from light for 10 minutes and check on a flow cytometer.

Ultrasound Examination

Experienced ultrasonographers performed an abdominal ultrasound on the patients. Unique to S. japonicum infection is parenchymal fibrosis, a network pattern that is often described as fish scale or tortoise shell-like. Hepatic fibrosis grading was carried out according to the standard of practical ultrasound diagnostic guidelines (1996) developed by the World Health Organization/Tropical Disease Research Organization. Patients were divided into two groups based on the presence or absence of liver fibrosis detected by abdominal ultrasound. Mild or non-fibrosis group: normal and thicker light spot type, normal liver sonogram or only thick liver parenchyma echo; moderate and severe fibrosis group: focal echoes in the liver parenchyma are scattered without clear boundary and echo density bands form a continuous network,fish-scale and cobweb type.[2, 29-32]

Statistical analysis

Medians were used as measures of central tendency. Comparisons between two groups were performed using the Mann–Whitney U test for non-normally distributed variables. The comparison between groups was evaluated using the Kruskal–Wallis test. Correlation analysis was performed using pointwise point biserial correlation analysis. All statistical analyses were performed using GraphPad Prism version 8.0 for Windows (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Demographic characteristics of the Study Population

Dongting Lake region in Hunan province is one of the most highly endemic for schistosomiasis japonica in history, but to now infection is under control. In this study, we included 135 patients with schistosomiasis from the Dongting Lake region in Changde City, Hunan province, including 84 with chronic schistosomiasis and 51 with advanced schistosomiasis. And 24 healthy volunteers were included in the control group. The demographic characteristics of different groups included in this study were shown in **Table 1** . The median age of infected participants and healthy volunteers was 58, 63 and 59 years old. No significant differences were observed between different groups.

Liver function abnormality in patients with advanced schistosomiasis

Liver function is closely related to the progression of schistosomiasis japonica. The results in patients with adequate laboratory tests of the hepatic profiles were shown in **Table 2** . It was observed that liver function varies among patients in different stages of schistosomiasis. Except for ALT and HDL, there were some differences between the two groups in other liver function-related markers, including TBIL, DBIL, ALB, and AST, and blood lipid related indicators, including TC, TG, and LDL. Patients with advanced schistosomiasis had a higher incidence of liver function abnormality and blood lipid than those with chronic schistosomiasis, which might be due to a longer history, more severe disease progression, and older age. The rate of liver function abnormality was significantly higher in patients with advanced schistosomiasis than that in those with chronic schistosomiasis (**Fig 1, Table 2**)

Lymphocytes in patients with different stages of schistosomiasis

The hematological profile can reflect the immune state to some extent. To evaluate the immune state, we first analyzed the blood routine data, which was shown in **Table 3** , particularly WBC, lymphocytes, and neutrophils. It was observed that hemoglobin, red blood cells, and platelets in the advanced group were significantly less than that in the chronic group. In addition, the number of lymphocytes was less in the advanced schistosomiasis patients when compared with those with chronic schistosomiasis. And the percentage of lymphocytes was also lower in the advanced group (**Fig 2**).

Peripheral Lymphocyte Subsets Between Patients at Different schistosomiasis Stages

Flow cytometry was used to compare immune cell subsets in the peripheral blood in patients at different stages of schistosomiasis (**Table 4**). The percentage of NK cells in patients with schistosomiasis increased when compared with the health control group, however, no significant difference was observed in different stages of schistosomiasis regarding the percentage of NK cells (**Fig 3D**). There were no differences in the percentages of $CD3^+$T cells and $CD8^+$T cells between the chronic group and the advanced group (**Fig 3A,3B**). However, the percentage of $CD4^+$T cells was lower in the advanced group, but the percentage of CD19+B cells was higher in the advanced group (**Fig 3C**). Also, the number of immune cells is of great significance. We noticed that the number of CD3+T cells, CD3+CD4+T cells, CD3+CD8+T cells, and NK cells were less in the advanced group when compared with those in the chronic group (**Fig 4A,4B,4C,4D**). Interestingly, no significant difference was detected in the number of $CD19^+$B cells in different stages of schistosomiasis japonica (**Fig 4E**).

Comparison of Peripheral Lymphocyte Subsets Between Patients with Different Disease Stages of Imaging

Based on the classification method described previously, we reclassified the patients with perfect clinical data based on imaging and divided the patients into three groups, 34 in mild group, 60 in fibrosis group. (**Table 5**) We found that the percentage and the absolute number of T cells, particularly $CD4^+$ T cells were indeed statistically different. T cells and $CD4^+$ T cells were negatively correlated with disease progression. No significant correlation was found for the percentage and the absolute number of each of the other peripheral lymphocytes.

Discussion

The immune state in the chronic stage of schistosomiasis infection is mainly mediated by granulomatous inflammatory responses to schistosome eggs deposited in different organs and tissues. Schistosoma japonicum mainly attacks the liver, and granuloma formation caused by egg deposition in the liver leads to periportal fibrosis, which will finally lead to the advanced stage of schistosomiasis japonica characterized by portal hypertension and hepatosplenomegaly[33]. Ascites and variceal hemorrhage are two serious and common complications at this stage, which can lead to the death of the patient. Although characteristics of immune cells peripheral in schistosomiasis mansoni was well defined, the features of peripheral immune state in patients with schistosomiasis japonica remain not well defined. In this study, we characterized the percentages and the absolute number of lymphocytes in the peripheral. We found that the percentage of CD4+T cells was lower in the advanced group, but the percentage of $CD19^+$B cells was higher in the advanced group.

In addition, the number of $CD3^+$T cells, $CD3^+CD4^+$T cells, $CD3^+CD8^+$T cells, and NK cells was less in the advanced group when compared with those in the chronic group. Our results indicated that the immune state in the peripheral may facilitate differential diagnosis of different stages of schistosomiasis japonica and they can be a potential prognostic factor.

Our study showed the differences in the liver function of the patients. Liver fibrosis and portal hypertension can be mediated by schistosomiasis[3]. Consistent with previous studies, we found advanced schistosomiasis is a wasting disease and can lead to a reduction in albumin in patients[34]. This is corroborated by the lower lipids in patients with advanced schistosomiasis. Also, differences in blood counts between patients at different stages of the disease have been confirmed. The reduction in various immune cells may be associated with hypersplenism, which is common in advanced schistosomiasis.

The peripheral immune state showed the potential to assist in the differential diagnosis of different stages of schistosomiasis[35]. It is well known that schistosomiasis infection is a process in which the immune status changes and the immunological diagnosis is widely used in the diagnosis of schistosomiasis[1, 36]. The conventional ELISA test has been widely used, but it can often only determine whether a patient is infected with schistosomiasis but cannot differentiate the disease progression of schistosomiasis.

Schistosome infection leads to significant changes in immune function in patients, with the Th1 response dominating in the acute phase, but gradually shifting to a Th2 response as the schistosomes mature over time[16, 18, 22, 37]. And it in turn gradually diminishes with the formation of chronic granulomas[16, 22]. Cytokines have received more attention in many studies, but direct immune cells have been less frequently mentioned[16]. Combining the cytokine profile and the immune cell profile will provide further help in understanding the immune state of patients with schistosomiasis.

Flow cytometry is a relatively convenient test, which can gives rapid results and provides more direct insight into the immune status of the patient. The use of peripheral lymphocytes alone as a biomarker to differentiate schistosomiasis progression may not be accurate, but we believe it has some value as an adjunctive diagnostic tool.

Immune status is different between the different stages of schistosomiasis. Patients with advanced schistosomiasis exhibit a higher percentage of haemocytopenia, which is related to hypersplenism in patients with advanced schistosomiasis. Splenectomy is one of the more commonly used and effective treatments for severe cases of this condition[13, 38-40]. The deterioration in liver function indicators is a direct indication of the more severe liver damage in patients with advanced schistosomiasis. If the markers mentioned in this article can be used to determine early that a patient with chronic schistosomiasis is progressing towards advanced schistosomiasis rather than remaining stable, and to guide early prevention and treatment, this can make a considerable difference to survival and quality of the life.

Abdominal ultrasound is also a common tool for screening for schistosomes, and the clinical impact of more severe and long-term infections often reveals fish-scale and cobweb changes in the liver, which are also referred to as "schistosomal liver". [29, 41]We reclassified the patients according to imaging and analyzed their peripheral lymphocyte status, which also suggested a correlation between $CD4^+$ T-cell percentage and liver fibrosis. As the patient's liver fibrosis worsened, the patient's $CD4^+$T-cell percentage subsequently decreased. This is also in line with the unique decline in $CD4^+$ T-cell percentage seen in advanced schistosomiasis that we mentioned before. Of course, not all patients presenting with the schistosomal liver are advanced schistosomiasis some of the most severely ill patients are chronic, and there is a proportion of patients who have not regressed to advanced schistosomiasis after years of persistent infection, but we do not think this affects our studies on peripheral lymphocytes in patients, which can still suggest the progression of disease in our patients.

In summary, Schistosoma japonicum patients at different stages of the disease differ in several indicators. $CD4^+$ T cells may be a potential biomarker that can assist in the differential diagnosis between chronic schistosomiasis and advanced schistosomiasis and may be a potential prognostic factor.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

We thank Professor Guanghui Ren for his insightful and thoughtful suggestions in this manuscript.

Table 1 Demographic of the study population.

Parameters	Chronic schistosomiasis (n=84)	Advanced schistosomiasis (n=51)	Healthy controls (n=24)	P-value
Age(years)	58(51-68)	63(56-70)	59(51-66)	ns
Gender (m/f)	66/26	37/19	18/6	ns

ns, not significant (p>0.05).

Table 2 Liver function of patients at different stages of the disease

Parameters	Chronic schistosomiasis (n=67)	Advanced schistosomiasis (n=43)	P-value
TBIL (umol/L)	14.6(11.3-16.95)	18(12.1-31.2)	0.0063
DBIL (umol/L)	3.4(2.35-4.5)	5(3.35-13.6)	0.002
ALB (g/L)	45.5(43.2-47.35)	39.6(34.35-44.95)	¡0.001
ALT (U/L)	24(19-29.5)	27.5(20.75-35)	ns
AST (U/L)	25(21-32)	35(27-49)	¡0.001
TC (mmol/L)	4.73(4.31-5.64)	3.91(3.32-4.83)	¡0.0001
TG (mmol/L)	1.32(0.90-1.76)	0.64(0.55-0.99)	0.0001
HDL (mmol/L)	1.19(1.04-1.44)	1.3(1.06-1.66)	ns
LDL (mmol/L)	3.31(2.73-3.95)	2.74(1.81-3.38)	0.0029

TBIL, total bilirubin; DBIL, direct bilirubin; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; TG, Triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ns, not significant (p>0.05).

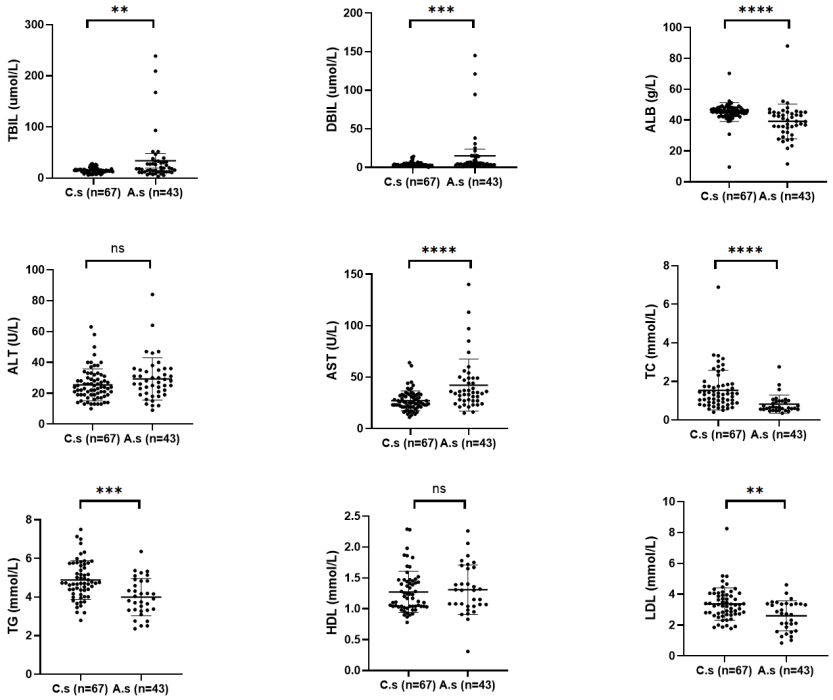

Figure 1

Liver function-related indicators in patients with different stages of schistosomiasis, showed a disparity in TBIL, DBIL, ALB, ALT, AST, TC, TG, HDL, and LDL between patients. C.s, chronic schistosomiasis, A.s, advanced schistosomiasis. *p <0.05, **p <0.01, ***p <0.005 (Mann-Whitney test).

Table 3 Blood count of patients at different stages of the disease

Parameters	Chronic schistosomiasis (n=67)	Advanced schistosomiasis (n=43)	P-value
WBC (10^9/L)	5.55(4.705-6.715)	4.8(3.495-5.78)	0.0053
Neutrophile (10^9/L)	3.12(2.695-4.21)	2.59(1.97-3.55)	ns
Lymphocyte (10^9/L)	1.76(1.405-4.05)	1.24(0.73-1.93)	0.0037 0.0267
Eosinophile (10^9/L)	0.13(0.08-0.265)	0.09(0.06-0.19)	
Neutrophile (%)	57.5(52.05-64.35)	60(50.95-71.4)	ns
Lymphocyte (%)	32(26.2-38.35)	29.2(18.7-36.45)	0.0414
Eosinophile (%)	2.3(1.5-4.05)	2.3(1.45-4.7)	ns
RBC (10^{12}/L)	4.54(4.155-5.04)	4.02(3.56-4.62)	0.0004
Hb (g/L) MCV (Fl)	141(128-152)	126(110.5-141.5)	0.0004 ns ns ns
MCH (pg) MCHC	93.55(90.68-97.93)	93.85(88.88-99.20)	
(g/Dl)	30.95(29.78-32.33)	30.7(28.38-32.93)	
	329(324-334)	327.5(319-334)	
Plt (10^9/L) MPV (Fl)	188(153.5-212.5)	113(78-184)	¡0.001 ns ns
PDW (%)	10.5(9.88-11.6)	11(10.1-11.7)	
	16.2(15.9-16.5)	16.2(15.8-16.8)	

WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Plt, platelet; MPV, mean platelet volume; PDW, platelet distribution width. ns, not significant (p>0.05).

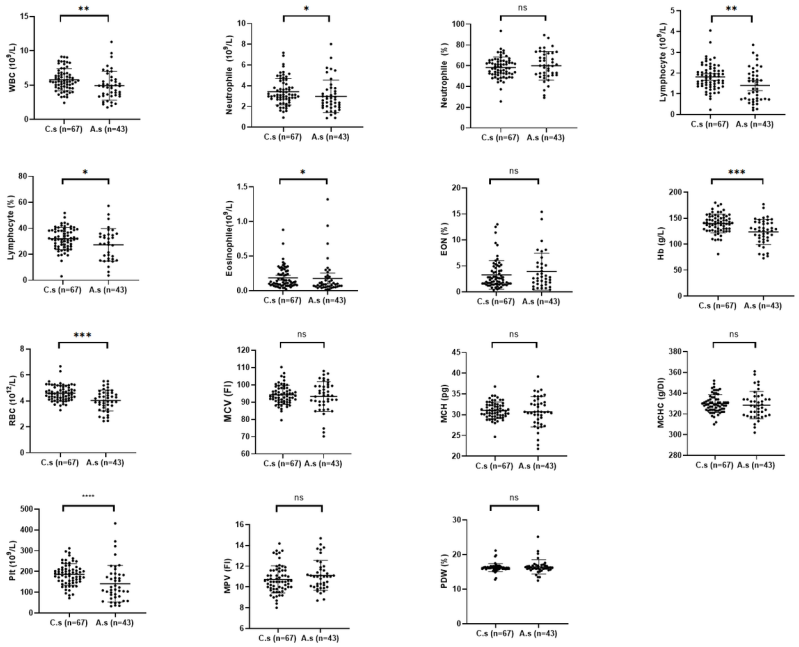

Figure 2

A graph reflecting blood routine tests in patients at different stages of schistosomiasis disease. Leukocytes and the difference between the percentage and absolute numbers of their subtypes (A-F). Differences in Hb, RBC, MCV, MCH, MCHC, Plt, MPV, and PDW of patients at different stages. *p <0.05, **p <0.01, ***p <0.005 (Mann-Whitney test).

Table 4 Comparison of Peripheral Lymphocyte Subsets Between Patients at Different Disease Stages

Parameters	Chronic schistosomiasis (n=84)	Advanced schistosomiasis (n=51)	Healthy controls (n=24)	P-value
T cells (%)	65.46(58.26-71.25)	60.85(47.25-68.60)	71.01(67.45-76.22)	0.003
$CD8^+$ T cells (%)	21.53(16.76-26.95)	20.38(15.795-24.08)	28.70(25.47-31.74)	¡0.001
$CD4^+$ T cells (%)	37.78(31.71-43.07)	33.55(25.26-42.32)	36.78(33.17-38.99)	ns
NK cells (%)	19.30(13.36-29.88)	20.51(12.52-31.08)	15.06(11.30-18.45)	0.0489
B cells (%)	10.44(7.99-13.34)	12.33(8.83-18.77)	11.05(8.96-14.37)	0.0393
T cells (cells/μL)	1079(824-1504)	614(479-1055)	1044(934-1346)	¡0.001
$CD8^+$ T cells (cells/μL)	360(267-521)	206(131-381)	482(385-530)	0.002
$CD4^+$ T cells (cells/μL)	630(484-835)	368(241-599)	575(432-678)	¡0.001
NK cells (cells/μL)	342(197-516)	189(103-465)	234(182-281)	0.0058

Parameters	Chronic schistosomiasis (n=84)	Advanced schistosomiasis (n=51)	Healthy controls (n=24)	P-value
B cells (cells/μL)	175(114-251)	133(102-228)	166(113-234)	ns

ns, not significant (p>0.05).

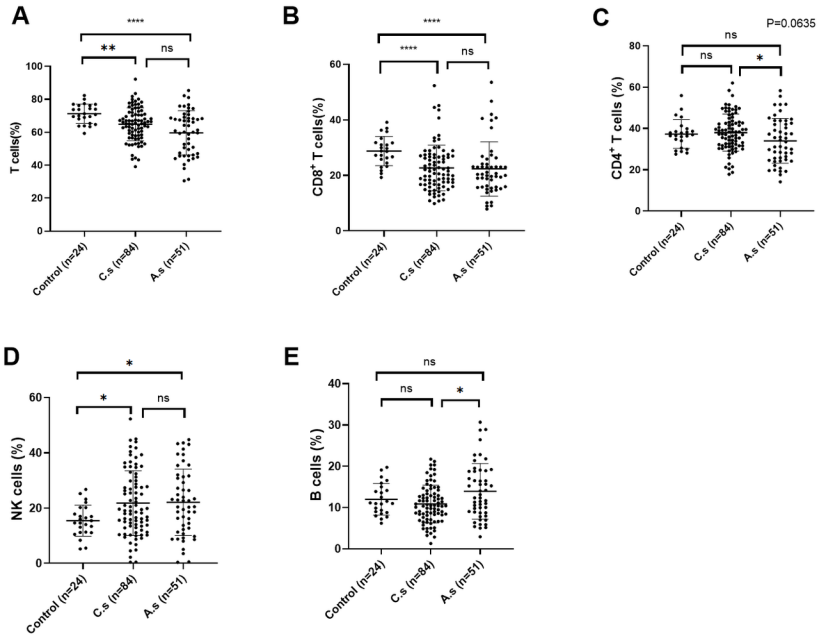

Figure 3

Representative plots of one experiment showing the percentage of $CD3^+$T cells(A), $CD8^+$T cells(B), $CD4^+$T cells(C), natural killer cells(D), and B cells(E) between the healthy control, chronic disease, and advanced disease groups. *p <0.05, **p <0.01, ***p <0.005, ns, not significant (p>0.05).

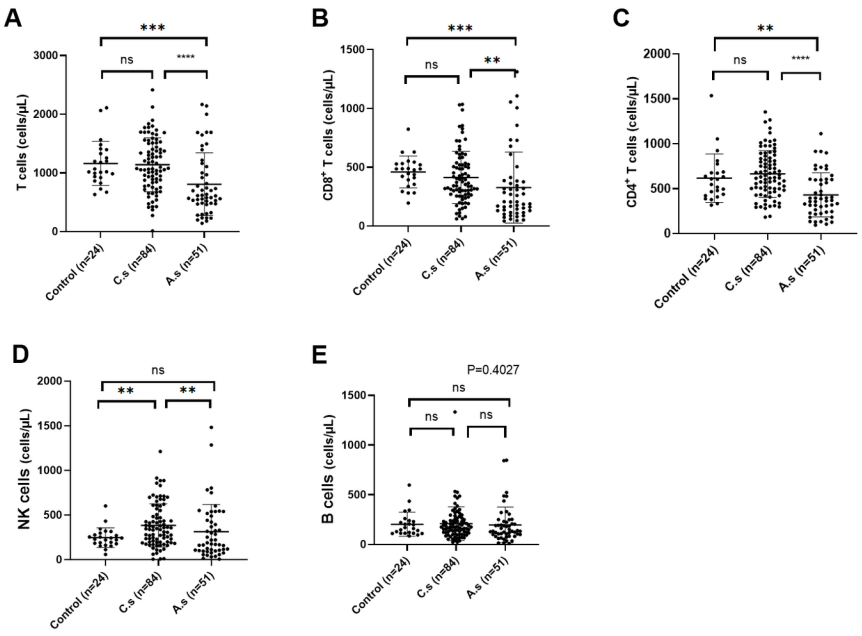

Figure 4

Representative plots of one experiment showing the absolute cell count of $CD3^+$T cells(A), $CD8^+$T cells(B), $CD4^+$T cells(C), natural killer cells(D), and B cells(E) between the healthy control, chronic disease, and advanced disease groups. *p <0.05, **p <0.01, ***p <0.005, ns, not significant (p>0.05).

Table 5 Analysis of peripheral lymphocyte status and its correlation in patients with different degrees of fibrosis under ultrasound examination

Parameters	Mild or non-fibrosis group (n=34)	Moderate and severe fibrosis group (n=60)	P-value	r-value
T cells (&)	66.48(60.94-70.94)	60.85(50.94-69.56)	0.0288	-0.2432
$CD8^+$ T cells (%)	22.71(16.14-27.68)	20.4(15.94-25.89)	ns	-0.006688
$CD4^+$ T cells (%)	38.74(33.34-47.96)	32.95(25.82-42.57)	0.0005	-0.2742
NK cells (%)	17.60(11.34-27.21)	22.71(13.79-34.72)	ns	0.1861
B cells (%)	11.27(8.96-14.29)	10.75(7.02-16.54)	ns	0.06258
T cells (cells/μL)	1054(699-1500)	752(530-1269)	0.0396	-0.1887
$CD8^+$ T cells (cells/μL)	332(200-517)	310(162-505)	ns	-0.04127
$CD4^+$ T cells (cells/μL)	610(443-818)	466(300-684)	0.0069	-0.2585
NK cells (cells/μL)	263(169-376)	283(124-540)	ns	0.08588
B cells (cells/μL)	176(122-222)	133(83-234)	ns	0.02458

ns, not significant (p>0.05).

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