ORIGINAL RESEARCH

Investigation of the Role of T-cell Type 17 Transcription Factor in Early Diagnosis of Cholangitis Disease

Tingting Cai, MMed; Yan Wang, MMed; Nan Peng, MMed; Lin Duan, MMed; Yang Yu, MMed

ABSTRACT

Context • Anti-mitochondrial antibody M2+ (AMA-M2+) primary bile cholangitis (PBC) is difficult to diagnose, and early diagnosis is the key to ensure effective treatment and the safety of patients.

Objective • The study intended to investigate the role of T helper type 17 (Th17) cells and their transcription factors in the early diagnosis of AMA-M2+ PBC to provide an effective guarantee of the ability to predict the prognosis of patients in the future.

Design • The research team designed a prospective controlled study.

Setting • The study took place at the Affiliated Hospital of Hebei University in Baoding, Hebei, China.

Participants • Participants were 30 patients with AMA-M2+ PBC at the hospital between November 2020 and August 2021 and 30 healthy controls who concurrently underwent physical examinations.

Outcome Measures • The study measured liver function (LF) and secretion of Th17 and its transcription factors—forkhead box P3 (Foxp3) and RAR-related orphan receptor gamma (RORγt)—and inflammatory factors—interleukin-17 IL-17 and IL-22—in participants' peripheral blood. The study also evaluated Th17 and its transcription factors in AMA-M2+ PBC, determined the expression of phosphorylated proteins using Western blotting, and analyzed the relationship between Th17 and LF.

Results • The Th17 in the intervention group's peripheral blood was significantly higher than that of the control group ($P < .05$), and the sensitivity and specificity of the AMA-M2+ PBC were 63.33% and 96.67%, respectively. The expression of Foxp3 and p-Foxp3 proteins for the intervention was significantly lower ($P < .001$), while RORγt and P-ROR γ T were significantly higher ($P < .001$). The levels of interleukin-17 (IL-17) and IL-22 for the intervention group were significantly higher than those for the control group. The Pearson correlation coefficient showed that alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ-glutamyl transpeptidase (GGT) were positively correlated with Th17 cells, RORγt, IL-17, and IL-22 and negatively correlated with Foxp3.

Conclusions • Th17 plays an important role in the early diagnosis of AMA-M2+ PBC, and Th17 and its transcription factors are highly effective for the early diagnosis of AMA-M2+ PBC, which is expected to be a breakthrough in the future diagnosis of the disease. (*Altern Ther Health Med.* 2023;29(2):168-173)

Tingting Cai, MMed, Physician; **Yan Wang, MMed**, Physician; **Lin Duan, MMed**, Physician; and **Yang Yu1, MMed**, Physician; Clinical Laboratory, the Affiliated Hospital of Hebei University, Baoding, Hebei, China. **Nan Peng, MMed,** Physician, School of Clinical Medicine, Hebei University, Baoding, Hebei, China.

Corresponding author: Yang Yu, MMed
E-mail: yuyang97@163.com

Primary biliary cholangitis (PBC) is an organ-specific, chronic, cholestatic, autoimmune liver disease with nonsuppurative inflammation of the intrahepatic bile duct as the main pathological change, which eventually leads to liver fibrosis and cirrhosis.[1] The disease has a predilection for middle-aged and older women, with a prevalence Worldwide of 155.8 out of 100 000 women over 40 years of age, showing a gradual rise year by year.[2]

At this time, the pathogenesis of PBC remains uncharacterized. The high level of early-stage latency results in medical practitioners having difficulty in making a clinical diagnosis as well as the inability to assess the occurrence of PBC in a timely manner.[3] The initial clinical manifestations are deceptive, with an increase in serum alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (GGT) as the only positive findings, and this delay can lead to a late diagnosis of cirrhosis in some patients.[4] Because of this, the prognosis for PBC isn't ideal, and the five-year mortality rate of patients with liver cirrhosis has reached about 60-70% in recent years.[5]

In addition, high-titer anti-mitochondrial antibodies (AMAs) can accompany PBC. AMA PBC, as a type of PBC with a faster development of more serious disease, urgently needs earlier diagnosis.[6] As an antibody against mitochondrial, inner-membrane, lipoprotein components, AMA has no organ or species specificity and can involve any of the five immunoglobulins, which are often used for auxiliary diagnosis of jaundice and liver diseases.[7]

Compared with ordinary PBC, AMA-M2 positive (AMA-M2+) PBC generally causes more significant autoimmune dysfunction and usually develops more rapidly and seriously. Approximately 70% of patients with AMA-M2+ PBC eventually develop cirrhosis, according to Shah and Kowdley's survey.[8] Similar to other autoimmune diseases, the severe immune disorder that AMA-M2+ PBC patients generally develop can cause immune-cell overproliferation that attacks normal intrahepatic bile ducts. In addition, although early-stage PBC has no obvious clinical symptoms, an immune overreaction has already begun in the body.[9]

T Helper Type 17 Cells

Yan et al's clinical research found that AMA-M2+ PBC is characterized by linkage with and a cascading immunoreactive loss of intrahepatic, bile-duct epithelial cells, in which various immune cells and cytokines play an extremely important part.[10] T helper type 17 (Th17) cells, as one of the essential immune cytokines in the human body, have attracted the attention of researchers, and exploring the involvement of Th17 in AMA-M2+ PBC is expected to bring about a breakthrough in the future diagnosis and treatment of the disease.

Damasceno et al found that Th17 might interfere with the onset and progression of AMA-M2+ PBC.[11] Th17 cells and their effectors can mediate defense mechanisms such as the host's response to various infections, especially extracellular bacterial infections, and participate in the pathogenesis of many autoimmune diseases.[12] A transcription factor mainly controls Th17 cell differentiation, and the depletion of the transcription factor can lead to a weakening of Th17 activity.[13]

Th17 and its transcription factors are essential in AMA-M2+ PBC, and the most important method of Th17's malignant influence on the human body is through the secretion of inflammatory factors.[14] The Th17 cells, which mainly secrete interleukin-17 (IL-17) and IL-22,[15] specifically produce interleukin-17 (IL-17) effectors that mediate inflammatory and autoimmune reactions.[16] IL-17, the prototype of the Th17 cytokine and the most important effector that Th17 cells secrete, participates in pro-inflammatory responses by effectively mediating neutrophils.[17] IL-22, produced by Th17 cells, plays a crucial part in mucosal surface host defense and tissue repair.[18]

The Th17 cells, are a subtype of CD4+ helper cells that aren't the traditional Th1 and Th2 subtypes, and the transcription factors are forkhead box P3 (Foxp3) and RAR-related orphan receptor gamma (RORγt).[19] Primary CD4+T cells can differentiate Foxp3 into RORγt at the early stage of the immune response, promoting Th17 cell differentiation.[20]

Yasuda et al found that disruption of the balance between pro-inflammatory, Th17 cells and inhibitory regulatory T (Treg) cells is a key factor in the pathogenesis of autoimmune diseases.[21] CD4+CD25+ Treg cells are an important subclass of regulatory T cells that have potent immunosuppressive action against both autoantigens and nonself-antigens, and they are vital in maintaining peripheral tolerance and preventing autoimmune diseases.[22]

Clinical Diagnosis of AMA-M2+ PBC

Currently, the clinical diagnosis of early AMA-M2+ PBC usually depends on ultrasounds, immunofluorescence screening, magnetic resonance cholangiopancreatography, endoscopic ultrasonography, and even genetic detection of hereditary cholestasis syndrome.[23] If research can confirm the potential role of peripheral-blood Th17 and its transcription factors in AMA-M2+ PBC, that confirmation may provide a novel direction and research breakthrough for the early accurate diagnosis and further treatment of AMA-M2+ PBC.

Current Study

At present, the relationship between Th17 cells and their transcription factors and AMA-M2+ PBC still lacks reliable research support. The current study intended to investigate the role of T helper type 17 (Th17) cells and their transcription factors in the early diagnosis of AMA-M2+ PBC to provide an effective guarantee of the ability to predict the prognosis of patients in the future.

METHODS

Participants

The research team designed a prospective controlled study. The study took place at the Affiliated Hospital of Hebei University in Baoding, Hebei, China. Potential participants were patients with AMA-M2+ PBC at the hospital between November 2020 and August 2021 and healthy controls who concurrently underwent physical examinations.

Potential participants were included in the study if they: (1) were aged >18 years; (2) had received a diagnosis of PBC; (3) had positive results from an AMA-M2 serological test; (4) had negative indicators of a hepatitis virus infection after testing; and (5) were at the pathological stage I or II; stage I indicates no obvious clinical symptoms with normal histology and liver function (LF), and stage II indicates no obvious clinical symptoms with some pathological liver manifestations.[24]

Potential participants were excluded from the study if they: (1) had a history of schistosomiasis infection, drug-induced hepatitis, or alcoholic hepatitis; (2) had cardio-cerebrovascular diseases, autoimmune defects, mental diseases, or neoplastic diseases; or (3) were pregnant or lactating.

All participants signed informed consent forms. The research team conducted the study following the requirements of the Declaration of Helsinki. At the same time, we went to the Clinical Trials Registry to register this experiment and received approval from our hospital's ethics committee.

Procedures

Groups. The research team assigned the patients with AMA-M2+ PBC to the intervention group and the healthy controls to the control group.

Liver function. The research team used an automatic biochemical analyzer (Beckman Coulter AU5800, Shanghai, China) to measure the ALT, ALP, and GGT to determine liver function.

Th17 cell count, Foxp3, p-Foxp3, RORγt, p-RORγt, IL-17, and IL-22. The research team obtained four milliliters of fasting venous blood from each participant during the morning hours and divided it into two parts: one for the count of Th17 cells in the peripheral blood, using flow cytometry, and the other for the determination of serum concentrations of Foxp3, phosphorylation-Foxp3 (p-Foxp3), RORγt, phosphorylation-RORγt (p-RORγt), IL-17, and IL-22 using Western blotting to evaluate the transcription factors and an enzyme-linked immunosorbent assay (ELISA) to evaluate the inflammatory factors after centrifugation.

For the Western blotting the research team: (1) transferred the total protein isolated from the serum to a polyvinylidene fluoride (PVDF) membrane using electrophoresis, (2) performed one hour of blocking with 4% defatted milk, and (3) performed subsequent overnight cultivation at 4°C with the primary antibodies (Abcam, Cambridge, Cambridgeshire, UK). The team added a second antibody (Abcam, Cambridge, Cambridgeshire, UK) after washing the membrane with tris-buffered saline (TBS) and Polysorbate 20 (TBST) the next day and analyzed the gray value of the protein bands using the Image J program (National Institutes of Health, Bethesda, Maryland, USA) after development with enhanced chemiluminescence (ECL). Beijing Solarbio Science & Technology (Beijing, China) supplied the ELISA kits. The process was in strict compliance with the manufacturer's instructions.

Outcome measures. The research team compared the intervention group's LF, peripheral-blood Th17 and its transcription factors—forkhead box P3 (Foxp3) and RAR-related orphan receptor gamma (RORγt)—and inflammatory factors—IL-17 and IL-22—to those of the control group. The team also: (1) determined the diagnostic value of AMA-M2+ PBC, (2) performed phosphorylated-protein quantification of transcription factors using Western blotting, and (3) analyzed the connection between Th17 and LF for AMA-M2+ PBC patients.

Outcome Measures

LF. This indicator is determined by the expression levels of ALT, ALP, and GGT. ALT reference values: 5-40 U/L (male), 5-35 U/L (female). ALP reference values: 45-120 U/L (male), 50-130 U/L (female), GGT reference values: 11-50 U/L (male), 7-32 U/L (female).

Th17 cell count. Th17 cell is a type of cell with pro-inflammatory ability. The more such cells are present, the more severe the patient's inflammatory response.

Th17 transcription factors. Higher expression levels of Foxp3, p-Foxp3, RORγt and p-RORγt indicated that Th17 cells were more transcriptionally competent and had a greater ability to promote inflammatory responses.

Th17 inflammatory factors. Higher levels of IL-17 and IL-22 expression indicate a more severe inflammatory response caused by Th17.

Correlation of Th17 factors with LF. The correlation between IL-17, IL-22 and ALT, ALP, and GGT was analyzed by Pearson correlation coefficient. When the r value was negative and $P < .05$, it indicated that the two data groups were negatively correlated (i.e., 1 group of data was elevated and the other group was decreased). When the r value is positive and $P < .05$, it indicates a positive correlation between the two groups of data (i.e., the data in 1 group are elevated and the data in the other group are also elevated).

Statistical Analysis

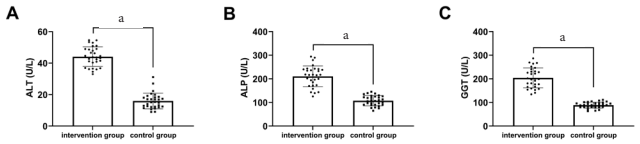
The research team used the SPSS22.0 software (IBM, Armonk, New York, USA) for the statistical analysis. The team analyzed: (1) the counting data, expressing it as N (%) and using the chi-square test and (2) the measurement data, expressing it as means ± standard deviations (SDs) and using an independent samples *t* test. The team visualized the diagnostic value using receiver operating characteristic (ROC) curves and performed a

Table 1. Comparison of Demographic and Clinical Characteristics of the Intervention and Control Groups at Baseline (N = 60)

	Intervention Group n = 30 Mean ± SD n (%)	Control Group n = 30 Mean ± SD n (%)	*t* or χ^2	*P* value
Age	45.87 ± 2.99	46.80 ± 2.98	1.223	.226
BMI, kg/cm^2	21.74 ± 3.78	21.49 ± 3.40	0.269	.789
Gender			0.218	.640
Male	2 (6.67)	3 (10.00)		
Female	28 (93.33)	27 (90.00)		
Family History of Illness			0.480	.488
Yes	6 (20.00)	4 (13.33)		
No	24 (80.00)	26 (86.67)		
History of Liver Disease			1.002	.317
Yes	7 (23.33)	4 (13.33)		
No	23 (76.67)	26 (86.67)		
Smoking			1.491	.222
Yes	9 (30.00)	5 (16.67)		
No	21 (70.00)	25 (83.33)		
Drinking			-	-
Yes	6 (20.00)	6 (20.00)		
No	24 (80.00)	24 (80.00)		
Place of Residence			1.086	.297
Urban	19 (63.33)	15 (50.00)		
Rural	11 (36.67)	15 (50.00)		
Nationality			2.069	.150
Han	28 (93.33)	30 (100.00)		
Minority	2 (6.67)	0 (.0..)		
Education Level			1.200	.273
Below high school	18 (60.00)	22 (73.33)		
High school and above	12 (40.00)	8 (26.67)		

Abbreviations: BMI, body mass index.

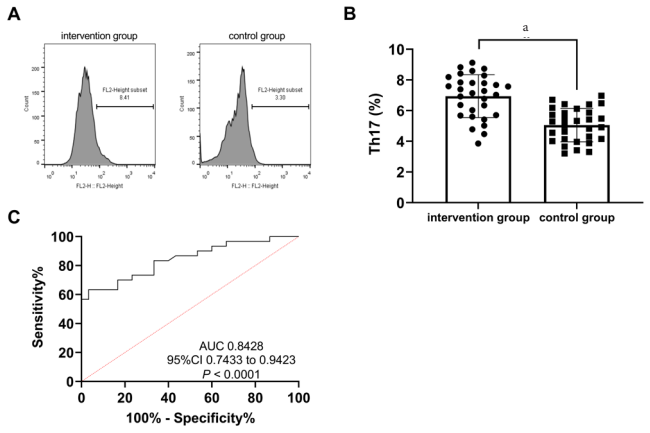
Figure 1. Comparison of Liver Function Between the Intervention and Control Groups. In the peripheral blood, Figure 1A shows the ALT; Figure 1B shows the ALP; and Figure 1C shows the GGT.



[a]$P < .001$, indicating that the ALT, ALP, and GGT were significantly higher in the intervention group than in the control group

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; Foxp3, forkhead box P3; GGT, γ-glutamyl transpeptidase.

Figure 2. The Level and Diagnostic Significance of Th17 cells in AMA-M2+ PBC for the Intervention and Control Groups. Figure 2A shows the results of flow cytometry experiments; Figure 2B shows the Th17 count; and Figure 2C shows the ROC curve for the diagnosis of AMA-M2+ PBC by Th17, as detected by Western blotting



[a]$P < .05$, indicating that the Th17 count was significantly higher in the intervention group than in the control group

Abbreviations: AMA-M2, anti-mitochondrial antibody M2; PBC, primary biliary cirrhosis; Th17, T helper type 17 cells.

correlation analysis using Pearson correlation coefficients. $P < .05$ indicated a statistically significant result.

RESULTS

Participants

The study included and analyzed the data of 60 participants, 30 in the intervention group and 30 in the control group (Table 1). The intervention group included 2 males and 28 females, with a mean age of 45.87 ± 2.99. The control group included 3 males and 27 females, with a mean age of 46.80 ± 2.98. No statistically significant differences existed between the groups' demographic and clinical characteristics at baseline ($P > .05$), suggesting comparability.

Liver Function

In the peripheral blood, the intervention group had a significantly higher: (1) ALT than the control group did (Figure 1A), at 44.10 ± 6.23 U/L and 15.93 ± 4.99 U/L, respectively ($P < .001$); (2) ALP than the control group did (Figure 1B), at 210.59 ± 44.25 U/L and 107.26 ± 21.43 U/L, respectively ($P < .001$); and (3) GGT than the control group did (Figure 1C), at 204.08 ± 41.92 U/L and 88.62 ± 12.44 U/L, respectively ($P < .001$).

Th17 Cell Count

The intervention group's Th17 count in the peripheral blood was 6.94 ± 1.40 (Figure 2A). That count was significantly higher than that of the control group (Figure 2B), at 5.05 ± 1.40 ($P < .05$). Figure 2C shows that the subsequent ROC curve analysis found, when the Th17 in the peripheral blood was >6.735%, that the sensitivity and specificity for diagnosing AMA-M2+ PBC was 63.33% and 96.67% for the intervention and control groups, respectively (data not shown.)

Th17 transcription factors

For the Western blot, Figures 3A and 3B shows that the intervention group's Foxp3 protein expression in the peripheral blood was significantly lower, at 0.51 ± 0.06, than that of the control group, at 0.62 ± 0.05 ($P < .001$). Figure 3C shows that the expression of the p-Foxp3 protein in the intervention group's peripheral blood was 0.39 ± 0.06, which was significantly lower than that of the control group, at 0.64 ± 0.05 ($P < .001$). Figure 3D shows that the expression of RORγt protein in the intervention group's peripheral blood was 1.05 ± 0.09, which was significantly higher than that of the control group, at 0.65 ± 0.05 ($P < .001$). Figure 3D shows that the expression of p-RORγt protein in the intervention group's peripheral blood was 1.06 ± 0.09, which was significantly higher than that of the control group, at 0.63 ± 0.05 ($P < .001$).

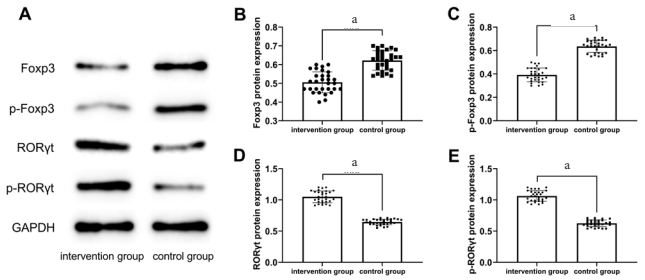
Th17 Inflammatory Factors

The intervention group's IL-17(Figure 4A) and IL-22 (Figure 4B) in the peripheral blood, at 1.05 ± 0.09 pg/mL and (0.65 ± 0.05 pg/mL), respectively, were significantly higher than those of the control group, at 21.10 ± 6.94 pg/mL and 9.05 ± 3.81 pg/mL, respectively ($P < .001$).

Correlation of Th17 factors with LF

Table 2 shows that the LF indexes ALT, ALP, and GGT had a positive correlation with the Th17 cells, RORγt, IL-17, and IL-22 ($P < .001$ for all), while the those indexes had a negative correlation with Foxp3 ($P < .001$ for all).

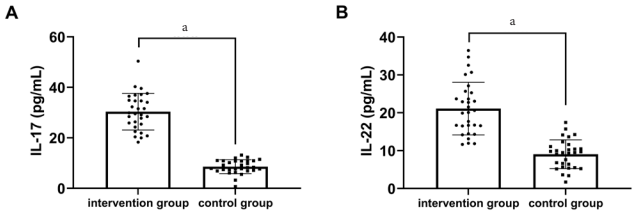
Figure 3. The Level and Diagnostic Significance of Transcription Factors Involved in Th17 Differentiation in AMA-M2+ PBC for the Intervention and Control Groups. Figure 3A shows the Western blot; Figure 3B shows the Foxp3 protein expression; Figure 3C shows the p-Foxp3 protein expression; Figure 3D shows the RORγt protein expression; and Figure 3E shows the p-RORγt protein expression



[a]$P < .001$, indicating that the intervention group's expression of the Foxp3 and p-Foxp3 proteins was significantly lower and of the RORγt and p-RORγt proteins was significantly higher than those of the control group

Abbreviations: AMA-M2, anti-mitochondrial antibody M2; Foxp3, forkhead box P3; PBC, primary biliary cirrhosis; p-Foxp3, phosphorylation-Foxp3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; p-RORγt, phosphorylation-RORγt; RORγt, RAR-related orphan receptor gamma; Th17, T helper type 17 cells

Figure 4. Comparison of Inflammatory Factors Secreted by Th17 for the Intervention and Control Groups. Figure 4A shows the IL-17, and Figure 4 B shows the IL-22 between two groups.



[a]$P < .001$, indicating that the IL-17 and IL-22 inflammatory factors were significantly higher in the intervention group than in the control group

Abbreviations: IL, interleukin; Th17, T helper type 17 cells.

Table 2. Correlation of Th17 Transcription Factors and Inflammatory Factors with LF (r/P)

	ALT	*P* value	ALP	*P* value	GGT	*P* value
Th17	0.587	<.001[a]	0.539	<.001[a]	0.614	<.001[a]
Foxp3	-6.412	<.001[a]	-0.574	<.001[a]	-0.671	<.001[a]
RORγt	0.642	<.001[a]	0.731	<.001[a]	0.751	<.001[a]
IL-17	0.633	<.001[a]	0.647	<.001[a]	0.597	<.001[a]
IL-22	0.642	<.001[a]	0.586	<.001[a]	0.804	<.001[a]

[a]$P < .001$, indicating that the ALT, ALP, and GGT had a positive correlation with the Th17 cells, RORγt, IL-17, and IL-22 and had a negative correlation with Foxp3

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; Foxp3, forkhead box P3; GGT, γ-glutamyl transpeptidase; IL, interleukin; LF, liver function; RORγt, RAR-related orphan receptor gamma; Th17, T helper type 17 cells.

DISCUSSION

The current study found a notably worse LF in the intervention group, as expected. An obviously elevated Th17 count in the intervention group indicated that Th17 might interfere with the onset and progression of AMA-M2+ PBC, which agrees with the results of Damasceno et al's previous study.[11]

The lower Th17 count in the intervention group compared to the control group in the current study validates Ang et al's findings that an immune overreaction can already have begun in the body in the early stages of disease.[16] Moreover, the current study found through the ROC analysis that Th17 was effective in diagnosing AMA-M2+ PBC, which carries huge clinical implications for an early diagnosis of AMA-M2+ PBC. For the evaluation of the influence of Th17 on AMA-M2+ PBC, the current study made a more comprehensive analysis of its specific mechanism in the human body. In Jiang T's study, they indicated that maintaining the balance of Treg/Th17 cells could alleviate the progression of cholangitis.[25] This is also consistent with our experimental results indicating that imbalance of Th17 cells will promote the malignant development of cholangitis.

The current study also confirmed that Th17 differentiation was notably enhanced in AMA-M2+ PBC, which once again emphasizes the connection between Th17 and AMA-M2+ PBC. Furthermore, alterations in the phosphorylation levels of Foxp3 and RORγt confirmed that view. The current study's ROC analysis also revealed that Foxp3 and RORγt had excellent results for the diagnosis of AMA-M2+ PBC, which further illustrates the important potential application value of Th17 in AMA-M2+ PBC.

In the current study, significant increases in IL-17 and IL-22 occurred in the AMA-M2+ PBC patients, suggesting that both of them had significant pro-inflammatory effects on the LF of the patients and accelerated their pathological development. However, due to too significant differences in IL-17 and IL-22 between the intervention and control groups, that finding doesn't have good reference significance for the results of the ROC analysis, so it' not further discussed results.

Finally, through correlation analysis, it was found that Th17 as well as its transcription factors and secreted inflammatory factors had obvious correlations with LF indexes of AMA-M2+ PBC patients, with synergistic action,

which further demonstrates the important significance of Th17 in evaluating AMA-M2+ PBC in the future.

However, due to the lack of experimental support in vitro, the current research team can't confirm the specific mechanism of Th17 in AMA-M2+ PBC. Patients with advanced AMA-M2+ PBC can be enrolled in future research to further evaluate the implications of Th17 in their pathological changes. The current research team will also conduct a long-term follow-up investigation on the participants of the current study to analyze the prognostic significance of Th17 in AMA-M2+ PBC patients.

CONCLUSIONS

Th17 plays an important role in the early diagnosis of AMA-M2+ PBC, and Th17 and its transcription factors are highly effective for the early diagnosis of AMA-M2+ PBC, which is expected to be a breakthrough in the future diagnosis of the disease.

AUTHORS' DISCLOSURE STATEMENT

The Baoding Science and Technology Planning Project, Grant No.2141ZF316, supported the study.

REFERENCES

1. Gulamhusein AF, Hirschfield GM. Primary biliary cholangitis: pathogenesis and therapeutic opportunities. *Nat Rev Gastroenterol Hepatol*. 2020;17(2):93-110. doi:10.1038/s41575-019-0226-7
2. Parés A. Primary biliary cholangitis. *Med Clín (Barc)*. 2018;151(6):242-249. doi:10.1016/j.medcli.2017.12.021
3. Prieto J, Banales JM, Medina JF. Primary biliary cholangitis: pathogenic mechanisms. *Curr Opin Gastroenterol*. 2021;37(2):91-98. doi:10.1097/MOG.0000000000000703
4. Gonzalez RS, Washington K. Primary biliary cholangitis and autoimmune hepatitis. *Surg Pathol Clin*. 2018;11(2):329-349. doi:10.1016/j.path.2018.02.010
5. Tanaka A. Current understanding of primary biliary cholangitis. *Clin Mol Hepatol*. 2021;27(1):1-21. doi:10.3350/cmh.2020.0028
6. Lindor KD, Bowlus CL, Boyer J, Levy C, Mayo M. Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases. *Hepatology*. 2019;69(1):394-419.
7. Lleo A, Leung PSC, Hirschfield GM, Gershwin EM. The pathogenesis of primary biliary cholangitis: A comprehensive review. *Semin Liver Dis*. 2020;40(1):34-48. doi:10.1055/s-0039-1697617
8. Shah RA, Kowdley KV. Current and potential treatments for primary biliary cholangitis. *Lancet Gastroenterol Hepatol*. 2020;5(3):306-315. doi:10.1016/S2468-1253(19)30343-7
9. Ang QY, Alexander M, Newman JC, et al. Ketogenic diets alter the gut microbiome resulting in decreased intestinal Th17 cells. *Cell*. 2020;181(6):1263-1275.e16. doi:10.1016/j.cell.2020.04.027
10. Yan JB, Luo MM, Chen ZY, He BH. The function and role of the Th17/Treg cell balance in inflammatory bowel disease. *J Immunol Res*. 2020;2020:8813558. doi:10.1155/2020/8813558
11. Damasceno LEA, Prado DS, Veras FP, et al. PKM2 promotes Th17 cell differentiation and autoimmune inflammation by fine-tuning STAT3 activation. *J Exp Med*. 2020;217(10):e20190613. doi:10.1084/jem.20190613
12. Wu B, Wan Y. Molecular control of pathogenic Th17 cells in autoimmune diseases. *Int Immunopharmacol*. 2020;80:106187. doi:10.1016/j.intimp.2020.106187
13. Fasching P, Stradner M, Graninger W, Dejaco C, Fessler J. Therapeutic potential of targeting the Th17/Treg axis in autoimmune disorders. *Molecules*. 2017;22(1):134. doi:10.3390/molecules22010134
14. Bunte K, Beikler T. Th17 Cells and the IL-23/IL-17 Axis in the pathogenesis of periodontitis and immune-mediated inflammatory diseases. *Int J Mol Sci*. 2019;20(14):3394. doi:10.3390/ijms20143394
15. Shan J, Jin H, Xu Y. Cell Metabolism: A new perspective on Th17/Treg cell imbalance in systemic lupus erythematosus. *Front Immunol*. 2020;11:1027. doi:10.3389/fimmu.2020.01027
16. Lee GR. The balance of Th17 versus Treg cells in autoimmunity. *Int J Mol Sci*. 2018;19(3):730. doi:10.3390/ijms19030730
17. Huang N, Dong H, Luo Y, Shao B. Th17 cells in periodontitis and its regulation by A20. *Front Immunol*. 2021;12:742925. doi:10.3389/fimmu.2021.742925
18. Moser T, Akgün K, Proschmann U, Sellner J, Ziemssen T. The role of TH17 cells in multiple sclerosis: therapeutic implications. *Autoimmun Rev*. 2020;19(10):102647. doi:10.1016/j.autrev.2020.102647
19. Kamali AN, Noorbakhsh SM, Hamedifar H, et al. A role for Th1-like Th17 cells in the pathogenesis of inflammatory and autoimmune disorders. *Mol Immunol*. 2019;105:107-115. doi:10.1016/j.molimm.2018.11.015
20. Yang P, Qian FY, Zhang MF, et al. Th17 cell pathogenicity and plasticity in rheumatoid arthritis. *J Leukoc Biol*. 2019;106(6):1233-1240. doi:10.1002/JLB.4RU0619-197R
21. Yasuda K, Takeuchi Y, Hirota K. The pathogenicity of Th17 cells in autoimmune diseases. *Semin Immunopathol*. 2019;41(3):283-297. doi:10.1007/s00281-019-00733-8
22. Beurel E, Lowell JA. Th17 cells in depression. *Brain Behav Immun*. 2018;69:28-34. doi:10.1016/j.bbi.2017.08.001
23. Corpechot C, Chazouillères O, Rousseau A, et al. A placebo-controlled trial of bezafibrate in primary biliary cholangitis. *N Engl J Med*. 2018;378(23):2171-2181. doi:10.1056/NEJMoa1714519
24. Cazzagon N, Floreani A. Primary biliary cholangitis: treatment. *Curr Opin Gastroenterol*. 2021;37(2):99-104. doi:10.1097/MOG.0000000000000708
25. Jiang T, Zhang HW, Wen YP, et al. 5-Aza-2-deoxycytidine alleviates the progression of primary biliary cholangitis by suppressing the FoxP3 methylation and promoting the Treg/Th17 balance. *Int Immunopharmacol*. 2021;96:107820. doi:10.1016/j.intimp.2021.107820