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Th17 cells over 5.9% at admission indicate poor prognosis in patients with HBV-related acute-on-chronic liver failure

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Abstract

Our previous study demonstrated that Th17 cells increased significantly in patients with hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF). However, their prognostic role in HBV-ACLF patients remains unknown.

Sixty-eight consecutive HBV-ACLF patients were enrolled in this cohort study. Th17 cells were examined using flow cytometry. Disease severity scores were assessed. ROC curves were used to evaluate the value in predicting prognosis. Survival was analyzed using Kaplan–Meier curves. Predictors of mortality were determined by regression analysis.

Th17 cells were significantly higher in HBV-ACLF patients compared to patients with chronic hepatitis B and normal controls (both $P < .001$). Also, Th17 cells were higher in nonsurviving HBV-ACLF patients than in surviving patients ($P = .014$). Th17 cells were positively correlated with CLIF-Consortium ACLF (CLIF-C ACLF) score ($r = 0.240$, $P = .048$). ROC curves showed that the frequency of Th17 cells had accuracy in predicting 90-day prognosis equivalent to MELD, MELD-Na and CLIF-C ACLF scores in HBV-ACLF ($P = .34$, $P = .26$, and $P = .15$, respectively). More importantly, the area under the ROC curve (AUROC) increased when Th17 cells were combined with MELD, MELD-Na or CLIF-C ACLF score than using Th17 cells alone ($P = .021$, $P = .006$, and $P = .023$, respectively). Kaplan–Meier analysis revealed that higher Th17 cells (≥5.9%) were closely associated with poor overall survival in HBV-ACLF ($P = .0086$). Additionally, multivariate regression analysis showed that the frequency of Th17 cells over 5.9% was an independent predictor of mortality (OR = 0.154, $P = .025$).

Circulating Th17 cells positively correlated with disease severity in HBV-ACLF. The frequency of Th17 cells over 5.9% could serve as a prognostic biomarker for HBV-ACLF patients.

Abbreviations: 95% CI = 95% confidence interval, ACLF = acute-on-chronic liver failure, AUROC = area under the ROC curve, CHB = chronic hepatitis B, CLIF-C = Chronic Liver Failure Consortium, HBV = hepatitis B virus, MELD = model for end-stage liver disease, NC = normal control, PTA = prothrombin time activity, ROC curve = receiver operating characteristic curve, Tbil = total bilirubin.

Keywords: acute-on-chronic liver failure, biomarker, hepatitis B virus, prognosis, Th17 cells

1. Introduction

Acute-on-chronic liver failure (ACLF) is characterized by acute deterioration of pre-existing chronic liver diseases and is associated with substantial short-term mortality due to the development of multiple organ failure.[1] In China, HBV-related ACLF accounts for the majority of ACLF cases due to the high prevalence of HBV infection.[2] An unclear understanding of the pathogenesis of HBV-ACLF and a lack of effective therapy result in extremely high mortality.[3] There is growing evidence that immune-mediated response plays a core role in the mechanism of HBV-ACLF.[1,2]

Th17 cell is a relatively new discovered subset of $CD4^+$ T-helper cell characterized by the production of interleukin-17 (IL-17) and has received increasing attention. Several lines of evidence have shown that Th17 cells are involved in the pathogenesis of different types of liver diseases, including viral hepatitis, alcoholic liver disease, nonalcoholic steatohepatitis and hepatocellular carcinoma (HCC).[4] It has been reported that circulating Th17 cells in chronic hepatitis B (CHB) patients positively correlate with disease severity and extent of hepatic injury. Therefore, it has been supposed that Th17 cells contribute to CHB progression.[5,6] Furthermore, our previous study and others' reports have demonstrated that Th17 cells increased significantly in HBV-ACLF patients compared to CHB patients and participated in the progression of HBV-ACLF.[7–9] Recently, Th17 cells were found to be associated with poor prognosis in HCC patients and colorectal cancer patients,[10,11] indicating that Th17 cells may act as a biomarker in predicting patients'

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prognosis. However, to the best of our knowledge, the prognostic value of Th17 cells in HBV-ACLF patients has not yet been investigated.

Recently, several prognostic scores have been established to predict the outcomes of HBV-ACLF patients,[12] such as MELD (model for end stage liver disease) score, MELD-Na score and CLIF-C (chronic liver failure consortium) ACLF score. However, these indicators were established using routine clinical parameters, while the immunological factors were not included. Considering that Th17 cells play a pathogenic role in the mechanism of HBV-ACLF, we hypothesized that a higher proportion of Th17 cells may indicate poor prognosis in HBV-ACLF patients. Thus, the chief aim of the current study was to evaluate the prognostic value of Th17 cells in HBV-ACLF patients.

2. Methods

2.1. Study design and patients

This retrospective cohort study used data from our previous study investigating the mechanism of HBV-ACLF. HBV-ACLF patients admitted to our department between April 2009 and August 2010 were enrolled. Inclusion criteria were based on published guidelines. Adult HBV-ACLF patients who were willing to participate and consented to the study were screened based on previously described criteria.[7,13] Briefly, ACLF was diagnosed based on the development of jaundice (total bilirubin (Tbil) $\geq$171 μmol/L), prothrombin time activity (PTA) $\leq$ 40% and along with at least one of the other criteria ($\geq$ grade 2 hepatic encephalopathy, ascites, spontaneous bacterial peritonitis or hepatorenal syndrome). Exclusion criteria were: evidence of other liver diseases, including autoimmune liver diseases, Wilson's disease, or cancer; co-infection with other hepatitis virus or HIV virus; treatment with artificial liver support or immunomodulatory drugs; history of drug or alcohol abuse; medical record of renal, cardiovascular, pulmonary diseases; and pregnancy. Cirrhosis was clinically diagnosed when a small and nodular liver was found on imaging tests including ultrasound, computerized tomography scans, or magnetic resonance imaging.[14] Each patient was treated with internal supportive treatment (i.e., glycyrrhizin, reduced glutathione, ademetionine, alprostadil, polyene phosphatidylcholine, plasma, or albumin transfusion if needed and antiviral therapy using nucleos(t)ide analogs if HBV-DNA was detectable). All HBV-ACLF patients were followed for at least 3 months. Patients' outcomes were recorded as survival or nonsurvival. Twenty-eight CHB patients and 16 healthy controls (NC) during the same period were enrolled as controls. CHB patients were diagnosed as those who displayed HBsAg positive more than 6 months and exhibited signs of hepatitis and abnormal liver function. Clinical assessment was performed at admission and prior to therapy. Peripheral blood was collected and analyzed immediately. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by our hospital's ethics committee (The third affiliated hospital of Sun-Yat-sen University). Written informed consent was obtained from each participant prior to the study.

2.2. Cell staining and flow cytometry

APC-conjugated CD8 antibody was purchased from BD Biosciences (San Jose, CA). PerCP-Cy5.5-conjugated CD3 antibody and phycoerythrin (PE)-conjugated anti-IL-17A were purchased from eBioscience (San Diego, CA). The same isotype of antibodies were used as controls. Fresh heparinized peripheral blood was incubated for 5 hours at 37°C with 5% CO_2, with phorbol 12-myristate 13-acetate (PMA, 20 ng/mL; Sigma-Aldrich, St. Louis, MO) and ionomycin (1 μg/mL; Sigma-Aldrich) in RPMI 1640 medium. Monensin (1.7 μg/mL; Sigma-Aldrich) was added during the first hour of incubation. Cells were then treated with Fix & Perm reagents (Invitrogen, Carlsbad, CA), and further permeabilized and stained with the intracellular IL-17A. Data were analyzed using FACSCalibur and CELLQUEST software (BD Biosciences) as previously described.[7,13]

2.3. Virological assessment and liver biochemical assays

Serum HBV markers (including HBsAg, HBeAg, HBeAb, and HBcAb) and alpha-fetoprotein (AFP) were investigated using the Elecsys system (Hoffmann-La Roche, Basel, Switzerland). HBV-DNA levels were quantitated with real-time quantitative PCR using ABI7300 (Applied Biosystems, Foster City, CA). The detection limit of HBV-DNA was 100 IU/mL. Liver biochemical assays were performed using an autoanalyzer (TBA-30FR, Toshiba, Tokyo, Japan). PTA was measured using an automatic hemostasis/thrombosis analyzer (STA compact, Holliston, MA).

2.4. Disease severity assessment

MELD score and MELD-Na score were used to assess disease severity.[15] Briefly, these scores were calculated as following. MELD score = $3.8 \times \ln$ (bilirubin [mg/dL]) + $11.2 \times \ln$ (INR) + $9.6 \times \ln$ (creatinine [mg/dL]) + $6.4 \times$ (etiology: 0 if cholestatic or alcoholic, 1 otherwise). MELD-Na score = MELD score − Na − $0.025 \times$ MELD $\times$ (140 − Na) + 140. The CLIF-C ACLF score, which was recently developed for classification and prognostic assessment of ACLF patients,[16] was additionally used to evaluate disease severity. CLIF-C ACLF score was calculated as follows: CLIF-C ACLF score = $10 \times [0.33 \times$ CLIF-OFs + $0.04 \times$ Age + $0.63 \times \ln$ (WBC count)-2].

2.5. Assessment of complications

Complete medical histories, physical examinations, and laboratory parameters were acquired for HBV-ACLF patients. Complications were monitored and diagnosed based on the following standards. Diagnosis of spontaneous bacterial peritonitis (SBP) was based on the following criteria: ascitic fluid polymorphonuclear count $\geq$ 250 cells/mm^3 with or without a positive culture; or ascitic fluid polymorphonuclear count $<$ 250 cells/mm^3 but with a positive culture (non-neutrocytic bacterascites). Hepatic encephalopathy (HE) was diagnosed according to West Haven criteria.[17] Criteria for diagnosing hepatorenal syndrome (HRS) were serum creatinine $>$1.5 mg/dL (133 μmol/L); 2) failure to improve in renal function after diuretic withdrawal and plasma volume expansion; and 3) lack of an identifiable cause for renal failure.[18] Pneumonia was defined by infiltrates on chest radiography and satisfying at least 2 of the following criteria: fever (temperature $\geq$ 38.3°C); leucopenia or leukocytosis (white blood cells counts $\leq 4 \times 10^9$/L or $\geq 12 \times 10^9$/L); and purulent tracheal secretions and the presence of rales or bronchial breath sounds on physical examination.[19]

2.6. Statistical analysis

Data were analyzed using SPSS version 20.0 (Chicago, IL) and expressed as frequencies, median, and range or as mean ± standard error. Differences in variables were analyzed using ANOVA and Student's *t* tests (for normally distributed data) or

Table 1

Characteristics of participants enrolled in the study.

Group	NC (n = 16)	CHB (n = 28)	HBV-ACLF (n = 68)
Gender, male	15	26	61
Age, years	39.7 ± 2.0	37.2 ± 1.5	41 (18–75)
ALT, U/L	23.06 ± 2.17	113 (27–1658)	149.5 (15–1986)
AST, U/L	22.93 ± 1.88	123 (39–751)	172.5 (39–3023)
Tbil, μmol/L	ND	65 (23.9–602.08)	506.2 (183.8–1301.7)
PTA, %	ND	85 (45–126)	30 (17–40)
ALB, g/L	ND	39.46 ± 0.73	35.68 ± 0.63
PLT, 10^9/L	ND	167 (58–413)	92.5 (26–250)
HBeAg positive	0	19	30
HBV-DNA, $\log_{10}$ IU/mL	ND	4.92 ± 0.18	4.84 (2.70–8.87)

Data are shown as means and standard error (for normally distributed data) or median and range (for non-normally distributed data).
ACLF = acute-on-chronic liver failure, ALT = alanine aminotransferase, ALB = albumin, AST = aspartate aminotransferase, CHB = chronic hepatitis B, HBV = hepatitis B virus, NC = normal control, ND = not determined, PLT = platelet counts, PTA = prothrombin time activity, Tbil = total bilirubin.

Kruskal–Wallis and Mann–Whitney *U* tests (for non-normally distributed data). Categorical data were analyzed using chi-squared test and Fisher's exact test. Correlation was evaluated by the Pearson test. ROC curves were used to evaluate the accuracy of predicting prognosis. The best cut-off level of Th17 cells in predicting prognosis was selected using the Youden index, a well-characterized objective method that maximized the sum of sensitivity and specificity.[20] Comparison of ROC curves was performed using DeLong test.[21] Survival was analyzed using Kaplan–Meier curves. The association of relevant variables with survival was investigated using multivariate logistic regression analysis using forward stepwise selection. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated. A 2-sided $P < .05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 68 consecutive HBV-ACLF patients who met the diagnostic criteria were enrolled. The median time of acquiring HBV (calculated from the first time of HBsAg positive to the date of admission) was 13 years (range, 2–30). The median age was 41 years (range, 18–75). Among them, 17 patients (25%) were clinically diagnosed with cirrhosis before enrollment. 45 patients received antiviral therapy using nucleos(t)ide analogs. During the follow-up period, 29 patients survived, while 39 patients died. Thus, the overall mortality rate was 57%. In addition, the mortality rate was lower in patients without cirrhosis (25/51, 49%) than in cirrhotic patients (14/17, 82%, $P = .044$). Baseline characteristics of participants were shown in Table 1. No significant differences existed among 3 groups in age ($P = .095$) or gender ratio ($P = .816$).

3.2. Th17 cells were significantly higher in HBV-ACLF patients independent of HBeAg presence

Th17 cells were detected by flow cytometry (Fig. 1). Th17 cells were significantly higher in HBV-infected patients than in NC group. Furthermore, Th17 cells were higher in HBV-ACLF patients (5.04 ± 0.27%) than CHB subjects (3.58 ± 0.26%, $P < .001$) and NC group (2.06 ± 0.18%, $P < .001$). Also, Th17 cells were higher in CHB subjects than NC group ($P < .001$;

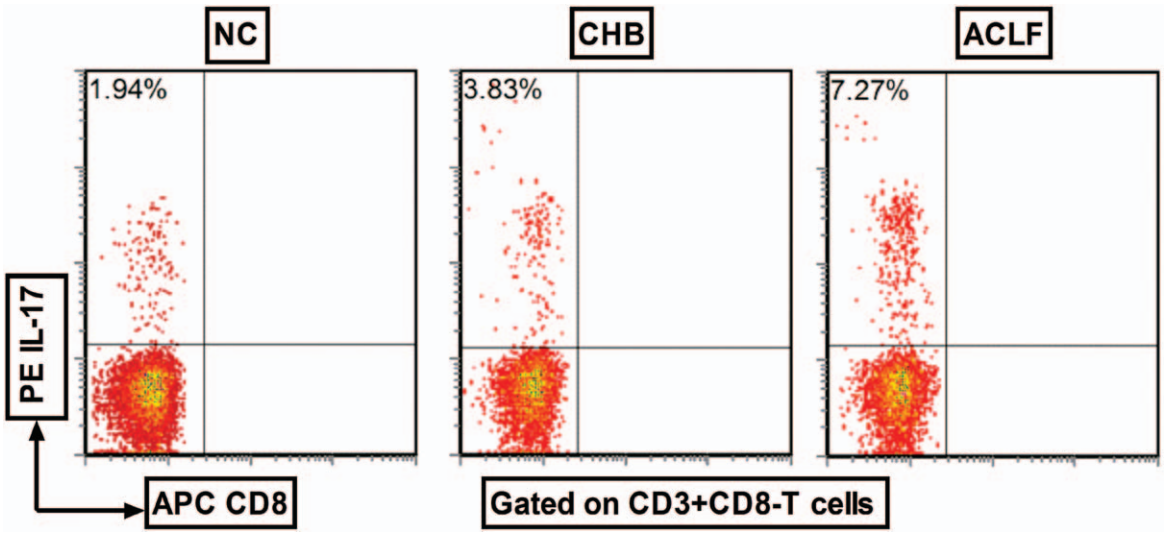

Figure 1. Th17 cells were stained and analyzed using flow cytometry. In this study, IL-17 + CD3 + CD8-T cells were defined as Th17 cells. Representative dotplots of IL-17 expression in peripheral CD3 + CD8- T cells of NC, CHB, and HBV-ACLF patients. The value in the upper left quadrant indicated the percentage of Th17 cells. ACLF = acute-on-chronic liver failure, CHB = chronic hepatitis B, HBV = hepatitis B virus, NC = normal control.

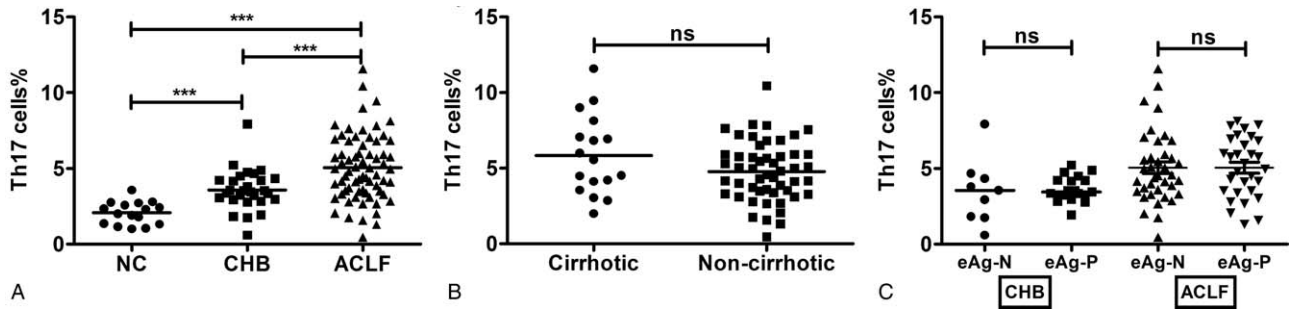

Figure 2. Th17 cells were significantly increased in HBV-ACLF patients independent of HBeAg presence. (A) Th17 cells were significantly higher in HBV-ACLF patients than in CHB and NC groups (both $P < .001$). (B) Th17 cells increased slightly in cirrhotic HBV-ACLF patients than noncirrhotic patients ($P = .085$). (C) No differences existed in Th17 cells between HBeAg-positive and HBeAg-negative patients in CHB group and HBV-ACLF patients. eAg-N, HBeAg-negative; eAg-P, HBeAg-positive; $^{***}P < .001$; ns, not significant. ACLF = acute-on-chronic liver failure, CHB = chronic hepatitis B, HBV = hepatitis B virus, NC = normal control.

Fig. 2A). Additionally, Th17 cells increased slightly in cirrhotic HBV-ACLF patients ($5.84 \pm 0.64\%$, n = 17) than noncirrhotic patients ($4.78 \pm 0.28\%$, n = 51, $P = .085$; Fig. 2B). We then determined the correlation between the presence of HBeAg and Th17 cells. In the CHB group, no significant difference existed in Th17 cells between HBeAg-positive ($3.63 \pm 0.19\%$, n = 19) and HBeAg-negative patients ($3.47 \pm 0.71\%$, n = 9, $P = .83$; Fig. 2C). Similarly, no significant difference was found in Th17 cells between HBeAg-positive HBV-ACLF patients ($5.04 \pm 0.36\%$, n = 30) and HBeAg-negative patients ($5.04 \pm 0.38\%$, n = 38, $P = .99$; Fig. 2C).

3.3. Th17 cells were closely correlated with disease severity in HBV-ACLF patients

MELD score, MELD-Na score and CLIF-C ACLF score are widely used to evaluate disease severity in HBV-ACLF patients. In this study, these parameters were calculated at admission. The results were 26.64 ± 0.53, 27.73 ± 0.52 and 42.02 ± 0.83 for MELD score, MELD-Na score and CLIF-C ACLF score, respectively. Next, correlations between Th17 cells and these parameters were examined. Interestingly, a positive correlation was found between Th17 cells and CLIF-C ACLF scores ($r = 0.240$, $P = .048$; Fig. 3A). Also, positive correlation trends were

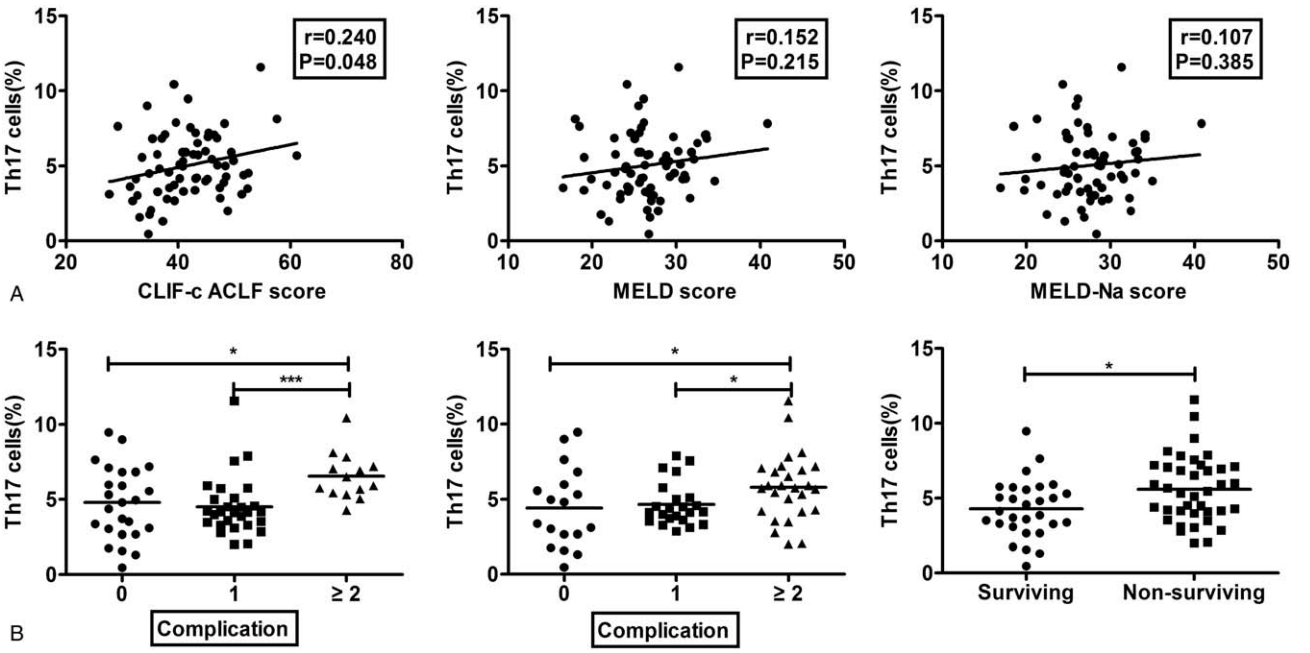

Figure 3. Th17 cells were closely associated with disease severity in HBV-ACLF patients. (A) CLIF-C ACLF score is recently developed to evaluate disease severity in HBV-ACLF patients. Th17 cells were positively correlated with CLIF-C ACLF score. Also, positive correlation trends were found between Th17 cells and MELD score, between Th17 cells and MELD-Na score. (B) At admission, Th17 cells were significantly higher in patients with at least 2 complications than in patients with one complication ($P < .001$) or without complication ($P = .025$). Similarly, during the whole hospital stay, patients with at least 2 complications had higher Th17 cells at admission than those with one complication ($P = .033$) and without complication ($P = .046$). Moreover, Th17 cells were significantly higher in nonsurviving patients than in surviving patients ($P = .014$). Collectively, these findings indicated that Th17 cells were closely associated with disease severity in HBV-ACLF. $^{*}P < .05$; $^{***}P < .001$.

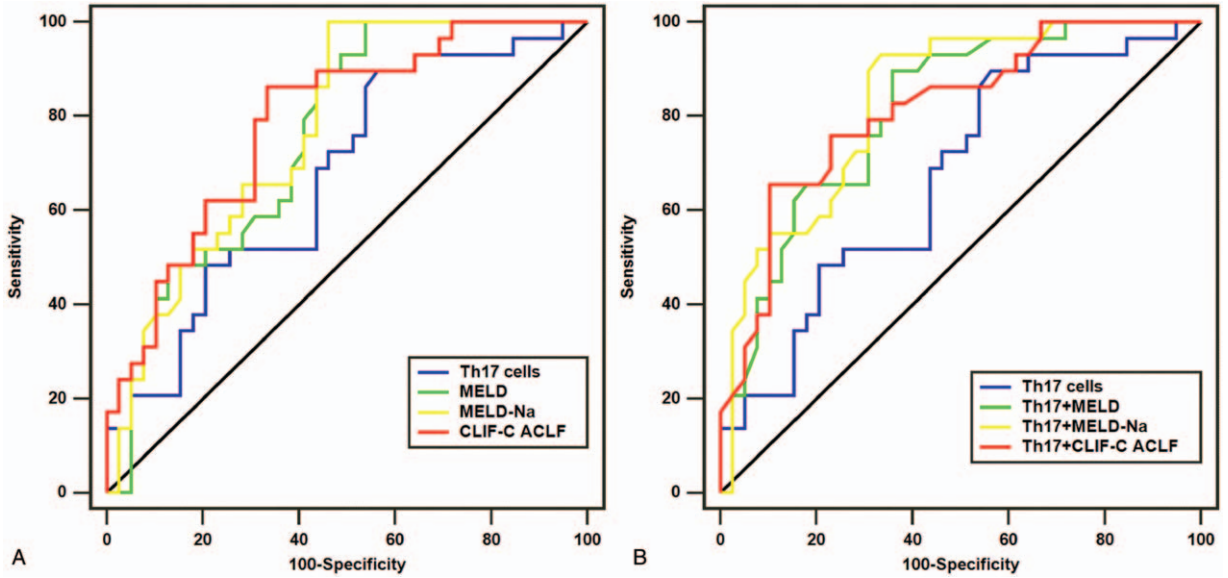

Figure 4. Higher Th17 cells at admission indicated poor prognosis in HBV-ACLF patients. The prognostic values of Th17 alone and in combination with updated prognostic parameters (MELD score, MELD-Na score and CLIF-C ACLF score) were assessed by ROC curves in HBV-ACLF patients. (A) ROC curves indicated that Th17 cells had accuracy of predicting prognosis in HBV-ACLF patients equivalent to MELD score ($P=.34$), MELD-Na score ($P=.26$) and CLIF-C ACLF score ($P=.15$). (B) Next, when Th17 cell levels were combined with MELD score, MELD-Na score and CLIF-C ACLF score, accuracy of predicting prognosis in HBV-ACLF patients was significantly increased than using Th17 cells alone ($P=.021$, $P=.006$ and $P=.023$, respectively).

found between Th17 cells and MELD scores (r = 0.152, $P=.215$), between Th17 cells and MELD-Na scores ($r=0.107$, $P=.385$; Fig. 3A). Traditionally, complications are assumed to be an important contributor to high mortality in HBV-ACLF patients. At admission, no complication existed in 26 patients, while 28 patients had one complication (23 patients with SBP and 5 patients with HE), and 14 patients had at least 2 complications (13 patients with SBP and HE; 1 patient with SBP, HE and HRS). Interestingly, Th17 cells were significantly higher in patients with at least 2 complications (6.54 ± 0.42%) than in patients with 1 complication (median 4.14%, $P<.001$) or without complication (4.80 ± 0.47%, $P=.025$; Fig. 3B). Complications were closely monitored during the whole hospital stay, and 23 new complications were detected (12 patients with HE, 8 patients with pulmonary infection, 2 patients with SBP and 1 patient with HRS). Similarly, patients with at least 2 complications had higher Th17 cells (5.80 ± 0.43%) at admission than those with one complication (median 4.16%, $P=.033$) and without complication (4.41 ± 0.62%, $P=.046$; Fig. 3B). Then, we examined the correlation between clinical outcome and Th17 cells at admission. Th17 cells were significantly higher in nonsurviving HBV-ACLF patients (5.60 ± 0.35%, n = 39) than in surviving patients (4.30 ± 0.36%, n = 29, $P=.014$; Fig. 3B). Collectively, these findings indicated that Th17 cells were closely associated with disease severity in HBV-ACLF.

3.4. Higher Th17 cells at admission indicated poor prognosis in HBV-ACLF patients

ROC curves were used to evaluate the ability of Th17 cells in predicting prognosis. The area under the ROC curve (AUROC) was 0.672 (95% CI: 0.547–0.781, $P=.0096$). The best cut-off level of Th17 cells in predicting prognosis was selected using the Youden index. By applying a cut-off point of 5.9%, the sensitivity was 89.66% (95% CI: 72.6–97.8%) and specificity was 43.59% (95% CI: 27.8–60.4%). Interestingly, no significant difference existed between AUROC values obtained using Th17 cells and those obtained with MELD score (0.756, $P=.34$), MELD-Na score (0.772, $P=.26$) or CLIF-C ACLF score (0.787, $P=.15$), indicating that Th17 cells at admission may have prognostic value equivalent to these scores (Fig. 4A). More importantly, the AUROC values increased when Th17 cells were combined with MELD score (0.809, $P=.021$), with MELD-Na score (0.834, $P=.006$) and with CLIF-C ACLF score (0.814, $P=.023$) than using Th17 cells alone in predicting prognosis (Fig. 4B). Collectively, these data suggested that Th17 cells at admission may be an effective predictor of prognosis for HBV-ACLF patients.

Patients were subsequently divided into 2 groups according to the cut-off value of 5.9%, a higher group (Th17 cells ≥5.9%, n = 22) and a lower group (Th17 cells <5.9%, n = 46). The 30-day survival rate was 70% (32/46) in the lower group, while it was 50% (11/22) in the higher group ($P=.178$). The 90-day survival rate was significantly higher in the lower group (25/46, 54%) than in the higher group (4/22, 18%, $P=.008$). In addition, 30-day and 90-day survival were examined using Kaplan–Meier analysis. The log-rank test revealed no significant difference in 30-day survival between the higher group and the lower group (Chi-square = 2.070, $P=.150$; Fig. 5A). However, a significant difference in 90-day survival was found between the higher group and the lower group (Chi-square = 6.906, $P=.0086$; Fig. 5B). These results indicated that higher Th17 cells were associated with poor overall survival.

3.5. Higher level of Th17 cells at admission was an independent predictor of mortality

Baseline clinical and laboratory variables were analyzed as possible predictors of survival. Basic characteristics of surviving and nonsurviving patients were summarized in Table 2. Nonsurviving

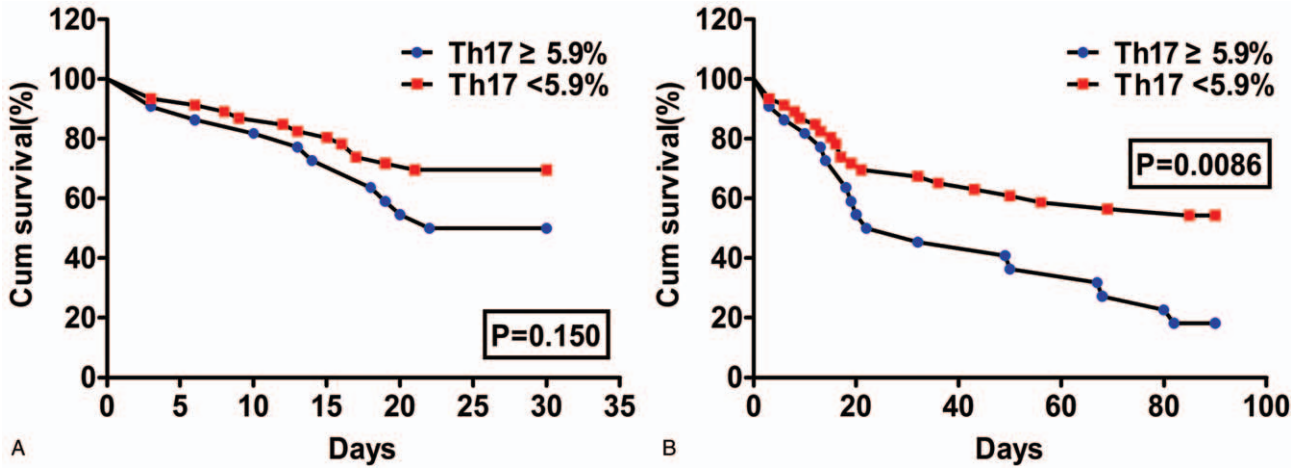

Figure 5. Th17 cells over 5.9% were associated with poor overall survival in HBV-ACLF. By applying a cut-off point of 5.9%, we divided HBV-ACLF patients into the higher group (Th17 cells ≥5.9%) and the lower group (Th17 cells < 5.9%). Survival was evaluated using Kaplan–Meier curves. (A) No significant difference was found in 30-day survival between the higher and the lower groups ($P = .150$). (B) Importantly, 90-day survival decreased significantly in the higher group than the lower group ($P = .0086$).

patients were older; were more likely to be female; had higher levels of Th17 cells, higher Tbil levels, and lower PTA; were more likely to be cirrhotic; and had more complications at baseline (Table 2). Next, logistic regression analysis was used to identify predictors of survival for HBV-ACLF patients. Age, cirrhosis, baseline complications, Th17 cells, Tbil level, PTA level, MELD score, MELD-Na score, and CLIF-C ACLF score were factors associated with a higher risk of mortality in HBV-ACLF patients according to univariate analysis (Table 3). Then we evaluated these significant variables in multivariate regression analysis using forward stepwise selection, only cirrhosis (OR = 0.060, $P = .015$), Th17 cells over 5.9% (OR = 0.154, $P = .025$), MELD score (OR = 0.741, $P = .011$) and CLIF-C ACLF score (OR = 0.829, $P = .005$) were found to be independent baseline predictors of survival in HBV-ACLF patients (Table 3).

4. Discussion

Although the pathogenic role of Th17 cells has been explored in several types of liver diseases, surprisingly less is known about their prognostic value in HBV-ACLF patients. To our knowledge, this is the first study to extensively examine the prognostic role of Th17 cells in a large-sample cohort study enrolling consecutive HBV-ACLF patients. We demonstrate for the first time that the frequency of Th17 cells was positively correlated with CLIF-C ACLF score. Moreover, using ROC curves, the accuracy of Th17

Table 2

Characteristics of HBV-ACLF patients based on clinical outcome.

Group	Survivors (n = 29)	Nonsurvivors (n = 39)	*P* value
Gender, male	29	32	.037
Age, years	38.90 ± 2.14	46.18 ± 2.09	.020
Cirrhosis	3	14	.023
Antiviral therapy	20	25	.797
Baseline complication (0/1/2, n)	17/8/4	4/11/24	<.001
MELD	24.46 ± 0.55	28.26 ± 0.73	<.001
MELD-Na	25.49 ± 0.57	29.40 ± 0.69	<.001
CLIF-C ACLF	38.12 ± 0.99	44.33 ± 1.01	<.001
Th17 cells, %	4.29 ± 0.36	5.60 ± 0.35	.014
PLT, 10^9/L	119 (29–249)	85 (26–250)	.079
Tbil, μmol/L	379.3 (193–957.9)	563 (183.8–1301.7)	.010
Dbil, μmol/L	238.1 (75.1–530)	337.6 (85–694.7)	.013
PTA, %	37 (23–40)	27 (17–40)	<.001
AST, U/L	160 (39–1154)	201 (46–3023)	.232
ALT, U/L	158 (15–1688)	141 (23–1986)	.958
ALB, g/L	36.42 ± 1.08	35.13 ± 0.76	.319
Na, mmol/L	138.5 (127.6–143.3)	137.2 (124.6–143.1)	.111
Cr, μmol/L	63 (44–102)	65.5 (34.5–124)	.500
AFP, ng/mL	79 (4–1000)	22.1 (5.6–1332.6)	.053
HBV-DNA, $\log_{10}$IU/mL	4.36 (2.70–7.44)	5.3 (2.70–8.87)	.074

Data are shown as means and standard error (for normally distributed data) or median and range (for non-normally distributed data).

ALT = alanine aminotransferase, ALB = albumin, AST = aspartate aminotransferase, CLIF-C = Chronic Liver Failure Consortium, Cr = creatinine, Dbil = direct bilirubin, HBV = hepatitis B virus, MELD = model for end-stage liver disease, PLT = platelet counts, PTA = prothrombin time activity, Tbil = total bilirubin.

Table 3

Factors associated with survival in HBV-ACLF patients.

Parameters	Wald	*P* value	OR	95% CI
Univariate analysis				
Age (years)	4.954	0.026	0.951	0.910–0.994
Gender (male)	<0.001	0.999	–	–
Cirrhosis	5.164	0.023	0.206	0.053–0.805
BL Complications	9.416	0.009		
One	4.601	0.032	0.294	0.096–0.900
⩾2	7.825	0.005	0.088	0.016–0.484
Th17 cells (%)	5.508	0.019	0.730	0.562–0.949
Th17≥5.9%	7.165	0.007	0.187	0.055–0.638
MELD	10.576	0.001	0.766	0.652–0.901
MELD-Na	11.114	0.001	0.751	0.634–0.889
CLIF-C ACLF	13.248	<0.001	0.820	0.737–0.913
Tbil (μmol/L)	6.252	0.012	0.997	0.995–0.999
PTA (%)	11.507	0.001	1.165	1.067–1.273
PLT (10^9/L)	2.802	0.094	1.008	0.999–1.018
AFP (ng/mL)	0.502	0.478	1.001	0.999–1.002
HBV-DNA, $\log_{10}$IU/mL	3.203	0.074	0.751	0.549–1.028
Multivariable analysis				
Cirrhosis	5.911	0.015	0.060	0.006–0.580
Th17⩾5.9%	5.027	0.025	0.154	0.030–0.790
MELD	6.475	0.011	0.741	0.588–0.933
CLIF-ACLF	7.949	0.005	0.829	0.728–0.945

95% CI = 95% confidence interval, ACLF = acute-on-chronic liver failure, BL = baseline, CLIF-C = Chronic Liver Failure Consortium, HBV = hepatitis B virus, MELD = model for end-stage liver disease, OR = odds ratio, PLT = platelet counts, PTA = prothrombin time activity, Tbil = total bilirubin.

cells in predicting prognosis was proved to be equivalent to MELD, MELD-Na and CLIF-C ACLF scores (all $P > .05$). When Th17 cells were combined with MELD, MELD-Na and CLIF-C ACLF scores, the accuracy of predicting prognosis was significantly increased than using Th17 cells alone (all $P < .05$). It is also the first demonstration that increased Th17 cells were associated with poor overall survival by Kaplan–Meier curves. And more importantly, the frequency of Th17 cells over 5.9% at admission was an independent predictor of mortality by multivariate regression analysis. Collectively, our data support the idea that Th17 cells over 5.9% indicate poor prognosis in HBV-ACLF patients.

It has been demonstrated that the Th17 cells pathway is involved in various liver diseases.[4] Niu et al[8] reported that intrahepatic IL-17$^+$T-cells were markedly increased in HBV-ACLF patients. Additionally, our previous study and others' results have revealed that peripheral Th17 cells were significantly higher in HBV-ACLF than in CHB patients.[7,9] However, these previous studies enrolled relatively small numbers of patients. In the present study, 68 consecutive HBV-ACLF patients were enrolled. To the best of our knowledge, the current study has recruited a larger sample of patients than all previous studies in determining the role of Th17 cells. Similarly, our study showed that circulating Th17 cell levels were significantly higher in HBV-ACLF patients than in CHB patients and normal controls (both $P < .001$). In addition, Th17 cells were significantly higher in nonsurviving HBV-ACLF patients than in surviving patients ($P = .014$). Moreover, the CLIF-C ACLF score, a newly updated prognostic score, has been found to be more effective and accurate in HBV-ACLF patients.[16,22] Interestingly, the frequencies of Th17 cells at admission were found to be positively correlated with CLIF-C ACLF scores ($P = .048$). Collectively, these findings indicated that Th17 cells may participate in the mechanism of HBV-ACLF.

Yang et al[23] reported that Th17 cell frequency was significantly associated with MELD score in HBV-ACLF. However, mild correlation between Th17 cell frequency and MELD score was observed in our study. This discrepancy might be complicated and could be patient related. In our study, more HBV-ACLF patients were enrolled than in Yang's report (68 vs. 44 patients), with a mean MELD score of 26.64 (range, 16.57–40.88). In addition, more deteriorated patients were enrolled in our study with higher total bilirubin levers than in Yang's report (506.2 vs 323.1 μmol/L, $P < .001$). Moreover, different examining times and viral loads may contribute to the difference. Thus, further studies are needed to elucidate the relationship between Th17 cells and MELD score in HBV-ACLF.

HBV-ACLF is commonly accompanied with many lethal complications, resulting in high mortality.[24] In this study, SBP and HE were the most common complications at admission and during the whole hospital stay. Patients with more complications at admission had higher Th17 cells than patients with fewer complications. The same trend was found for complications recorded during the hospital stay. These data indicated that increased frequencies of Th17 cells were closely associated with the development of complications.

Recently, several reports have revealed that Th17 cells could be used as a prognostic biomarker in cancer patients. Liao et al[25] reported that high expression of intra-tumoral IL-17 was associated with poorer survival and increased recurrence of hepatocellular carcinoma (HCC). Likewise, Yan et al[10] reported that intra-tumoral IL-17-producing cells were associated with overall survival and disease-free survival of HCC patients. A more recent study confirmed a high RORγT/CD3 ratio as a strong prognostic marker for postoperative survival, and Th17 cells may affect lymph node metastasis in colorectal cancer.[11] However, little is known about the prognostic value of Th17 cells in HBV-ACLF patients. In this study, we provide evidence for the first time that increased Th17 cells at admission predict poor prognosis in HBV-ACLF patients. First, Th17 cells were significantly higher in nonsurviving patients than in surviving patients ($P = .014$). ROC curves demonstrated that the accuracy of Th17 cells in predicting prognosis was equivalent to MELD, MELD-Na and CLIF-C ACLF scores (all $P > .05$). A value of 5.9% was subsequently chosen as the best cut-off value for Th17 cells using the Youden index. 90-day survival was significantly lower in patients with high Th17 cells than those with lower Th17 cells ($P = .0086$). In addition, the frequency of Th17 cells over 5.9% was proved to be an independent factor by multivariate logistic regression analysis ($P = .025$). Taken together, these data strongly indicated that Th17 cells at admission may be a potential prognostic factor in HBV-ACLF patients.

Due to the complicated and unclear mechanisms of HBV-ACLF, useful and effective prognostic factors are rare.[26,27] In the present study, when Th17 cells were combined with MELD, MELD-Na, and CLIF-C ACLF scores, the accuracy of predicting 90-day survival in HBV-ACLF patients was significantly increased than using Th17 cells alone (all $P < .05$). These results indicated that Th17 cells combined with updated prognostic scores can improve the accuracy of predicting patients' outcome. For those patients with Th17 cells over 5.9% at admission, internal supportive medicine failed in more than 80% of patients. Liver transplantation should be the only definitely effective therapy for these cases. However, because of the shortage of liver donors and high medical costs, liver transplantation is unavailable for all HBV-ACLF patients. Hence, early prediction of a lethal prognosis is important. Using the Th17 cells as an indicator

can help physicians recognize higher-risk HBV-ACLF patients earlier.

This study provided some unique findings compared to previous studies. First, the current study investigated the relationship between Th17 cells and the recently updated prognostic score (CLIF-C ACLF score). We demonstrated that Th17 cells at admission provided comparable accuracy to CLIF-C ACLF score in predicting prognosis for HBV-ACLF patients. Since CLIF-C ACLF score is considered superior to MELD score and MELD-Na score for stratifying HBV-ACLF patients based on risk of mortality, our study provided convincing evidence of the prognostic value of Th17 cells. Second, to our knowledge, the current study is the first report providing an optimal cut-off value for Th17 cells, which is very vital as a biomarker in clinical practice. Th17 cells over 5.9% at admission were considered to be a good candidate and more practical for HBV-ACLF prognosis in the current study. Also, we should note that our study was a pilot observational study, and the prognostic value of Th17 cells should be validated in clinical practice in the future.

In conclusion, our results suggest that Th17 cell level could be used as a promising biomarker to stratify patients based on risk and predict prognosis in HBV-ACLF patients, helping physicians with early identification of patients and choice of optimal treatments.

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Author contributions

Geng-lin Zhang, Ting Zhang and Zhi-liang Gao designed the experiments; Geng-lin Zhang, Ting Zhang, Qi-yi Zhao, and Chao-shuang Lin performed the experiments; Geng-lin Zhang and Ting Zhang collected and analyzed the data; Geng-lin Zhang and Ting Zhang wrote the main manuscript text. All authors reviewed the manuscript.

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