The Transforming Growth Factor β1/Interleukin-31 Pathway Is Upregulated in Patients with Hepatitis B Virus-Related Acute-on-Chronic Liver Failure and Is Associated with Disease Severity and Survival

Xueping Yu, a Ruyi Guo, a Desong Ming, b Yong Deng, c Milong Su, b Chengzu Lin, a Julan Li, a Zhenzhong Lin, b Zhijun Su a

Department of Infectious Diseases, The First Hospital of Quanzhou Affiliated to Fujian Medical University, Quanzhou, China a; Department of Clinical Laboratory, The First Hospital of Quanzhou Affiliated to Fujian Medical University, Quanzhou, China b; Department of Infectious Diseases, The Second People’s Hospital of Pingxiang, Pingxiang, China c

The transforming growth factor β1/interleukin-31 (TGF-β1/IL-31) pathway plays an important role in the process of cell injury and inflammation. The purpose of this work was to explore the role of the TGF-β1/IL-31 pathway in the cytopathic process of hepatitis B virus (HBV)-related acute-on-chronic liver failure (ACLF). The quantitative serum levels of TGF-β1, IL-9, IL-10, IL-17, IL-22, IL-23, IL-31, IL-33, and IL-35 were analyzed among chronic hepatitis B (CHB) patients (n = 17), ACLF patients (n = 18), and normal control (NC) subjects (n = 18). Disease severity in patients with ACLF was assessed using the model for end-stage liver disease (MELD) and Child-Pugh scores. Serum TGF-β1 levels were strongly positively correlated with IL-31 in all subjects, and both of them were positively correlated with IL-17, IL-22, and IL-33. In CHB and ACLF patients, serum levels of TGF-β1 and IL-31 were both increased significantly compared with those in NC subjects and positively correlated with total bilirubin (TBil) and alpha-fetoprotein (AFP) levels. ACLF patients showed the highest levels of TGF-β1 and IL-31, which were positively correlated with Child-Pugh scores. Furthermore, the recovery from the liver injury in CHB was accompanied by decreased TGF-β1 and IL-31 levels. More importantly, serum levels of TGF-β1 and IL-31 were markedly upregulated in ACLF nonsurvivors, and IL-31 displayed the highest sensitivity and specificity (85.7% and 100.0%, respectively) in predicting nonsurvival of ACLF patients. Increasing activity of the TGF-β1/IL-31 pathway is well correlated with the extent of liver injury, disease severity, and nonsurvival of ACLF patients, while reducing activity is detected along the recovery from liver injury in CHB, suggesting its potential role in the pathogenesis of liver injury during chronic HBV infection.

Hepatitis B virus (HBV)-related acute-on-chronic liver failure (ACLF) is triggered mainly by severe extensive liver injury, and the exact mechanisms of massive destruction of HBV-infected hepatocytes remain unclear. However, one of the current assumptions is that the imbalance of the cytokine network, the so-called cytokine storm theory (1), points to potential involvement of inflammatory cytokines in destroying the HBV-infected cells, which may provide an explanation for the aggravation of liver injury.

Transforming growth factor-β1 (TGF-β1) is a 25-kDa homodimeric protein composed of two subunits linked by a disulfide bond and is a powerful inhibitor of DNA synthesis and cellular proliferation (2). It also mediates formation of extracellular matrix and facilitates cell differentiation (3). Previous studies have shown that TGF-β1 plays a role in developing liver failure (LF). Miwa et al. found that the mRNA and protein expression of TGF-β1 were significantly upregulated in both the plasma and liver tissue in patients with fulminant liver failure (FLF) (4). Yoshimoto et al. found that the overexpression of TGF-β1 delayed liver regeneration and promoted perisinusoidal fibrosis and hepatocyte apoptosis in the rat model of FLF (5).

Interleukin-31 (IL-31), is a newly discovered proinflammatory cytokine and is produced mainly by CD4+ T cells, especially when cells are skewed toward a Th2 phenotype (6). It acts through the oncostatin receptor (OSMR) and heterodimeric receptors of IL-31 (IL-31R), a complex that stimulates the JAK-STAT, the phosphoinositol 3-kinase (PI3K)/AKT, and the RAS/extracellular signal-regulated kinase (ERK) signal pathways (7, 8). There is emerging evidence showing that the IL-31/IL-31R signaling pathway plays an important role in the pathogenesis of atopic and allergic diseases and inflammatory diseases such as allergic contact dermatitis (9, 10), nonatopic eczema (11), spontaneous urticaria (12), nasal polyps (13), asthma (14), and familial primary cutaneous amyloidosis (15). Nevertheless, there is a paucity of data exploring the potential role of IL-31 in the pathogenesis of ACLF.

Biological functions of TGF-β1 depend on the signal transduction and regulation of Smad proteins. Smad2/3 are the key elements in mediating TGF-β1-induced inflammatory diseases (16). Ge et al. (17) found that TGF-β1 induced Smad2 phosphorylation and blockade of Smad2/3 prevented TGF-β1-modulated IL-6 in-
crease. Activated Smad2 can bind to the IL-6 promoter region, including IL-31, a new member of the IL-6 family (17). Shi et al. also found that TGF-β1 induced Smad2 phosphorylation and then activated the binding of Smad3 to IL-31 promoters before finally stimulating the IL-31-JAK-STAT signal pathway (18). Therefore, IL-31, which increased with elevated TGF-β1 expression, was considered a downstream molecule of the TGF-β1–Smad2/3 pathway (18). Recently studies have shown that the TGF-β1–Smad2/3/IL-23 pathway plays an important role in the progression of bleomycin-induced pulmonary fibrosis in mice (18, 19), suggesting that the TGF-β1/IL-31 and Acute-on-Chronic Liver Failure relation to the pathological process of many human diseases. We decided to investigate the TGF-β1–Smad2/3/IL-23 pathway in ACLF because the pathogenesis of massive liver injury and lead to new treatment strategies.

In this study, we analyzed the serum levels of TGF-β1, IL-9, IL-10, IL-17, IL-22, IL-23, IL-31, IL-33, and IL-35, and investigated their relationships with the disease severity and survival in patients with ACLF and chronic hepatitis B (CHB) to address the potential role of the TGF-β1/IL-31 pathway in liver injury of ACLF and to build a foundation for identifying new therapeutic targets.

### MATERIALS AND METHODS

#### Subjects

Blood samples were collected on the next morning after admission from 17 CHB patients and 18 ACLF patients who were hospitalized or followed up from July 2012 to November 2013 in the Department of Infectious Diseases of The First Hospital of Quanzhou Affiliated to Fujian Medical University. The criteria for diagnoses of CHB and ACLF have been described in a previous study (20–25). All CHB patients who had positive hepatitis B surface antigen detection (HBsAg) and hepatitis-related clinical manifestations or a histological confirmation of hepatitis and abnormal alanine aminotransferase (ALT) levels (>40 U/liter) for more than 6 months were included. The diagnostic criteria for ACLF include mainly a history of CHB or liver cirrhosis, serum total bilirubin (TBil) 10 times or more than the normal level (>171 µmol/liter), and prothrombin time activity (PTA) of <40%. All participants were enrolled following the order of patient hospital admission, and there was no exclusion based on gender. The predominance in male patients most likely reflects the demographic features, where the majority of patients with advanced or end-stage liver disease are males.

No patients had received antiviral therapy or immunomodulating agents, such as glucocorticoid hormones and thymosin, before enrollment. Patients with hepatitis A, hepatitis C, hepatitis D, or human immunodeficiency virus (HIV) infection and those with alcohol-induced hepatitis or drug-induced autoimmune liver diseases and hepatic carcinoma (HCC) were excluded. Fresh blood samples from 18 healthy individuals without noticeable or detectable cell injury were designated normal controls (NC). Clinical characteristics of the enrolled cohorts are listed in Table 1.

Eighteen ACLF patients were divided into two groups by their final clinical outcome. The survival group (n = 11) included patients whose liver function and blood coagulation were recovered gradually within 6 months after blood sampling and were still alive by the completion of this study. The nonsurvival group (n = 7) included patients whose liver function deteriorated progressively and who died within 6 months after blood sampling. The details of the two groups have been described in a previous study (25). The clinical characteristics of the two groups were similar, with the exception of the international normalized ratio (INR) and model for end-stage liver disease (MELD) scores, which were higher in the nonsurvival group than in the corresponding survival group. Regrettably, we were unable to obtain liver biopsy specimens from ACLF patients because of their fragile clinical status and therefore could not perform immunohistochemical analysis of the liver inflammation and injury.

All patients with CHB signed a written informed consent form before they were treated with nucleos(t)ide analogs (entecavir, tenofovir, lamivudine, or adefovir dipivoxil), and none of them received glucocorticoid therapy. Only data from 12 CHB patients who were followed up for 5.3 ± 1.9 months were assessed in this study, and 9 of them were males with an average age of 35.8 ± 9.8 years old, ranging from 23 to 54 years old. There were 9 patients who received entecavir (Sino-American Shanghai Squibb Pharmaceuticals Ltd.) (0.5 mg/day orally), 2 patients were treated with lamivudine (GSK, Tianjin, China) (100 mg/day orally), and 1 patient was treated with tenofovir (Novartis Pharmaceutical Co., Ltd.) (600 mg/day orally).

The MELD score was calculated as 9.57 × ln serum creatinine (Cr) + 3.78 × ln serum total bilirubin (TBil) + 11.2 × ln international normalized ratio (INR) + 6.43. The Child-Pugh score was calculated using two clinical variables, ascites and encephalopathy, and three laboratory parameters, serum TBil, Cr levels, and prothrombin time (PT). The study protocol was approved by the Ethics Committee of The First Hospital of

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**TABLE 1 Clinical characteristics of the subjects enrolled in the study**

| Characteristic             | Value for group | P value<sup>c</sup> |
|----------------------------|-----------------|---------------------|
| No. (%) male               | NC (n = 18)     | CHB (n = 17)        | ACLF (n = 18)      |
| Age (yr)                   | 30 (23–50)      | 33 (23–49)          | 37 (21–57)         |
| No. (%) HBeAg positive     | ND              | 10 (58.5)           | 11 (61.1)          |
| HBV DNA (log copies/ml)    | ND              | 6.1 (4.7–6.9)       | 6.5 (4.2–7.1)      |
| ALT (IU/liter)             | ND              | 231.0 (60.4–950.6)  | 415.5 (52.0–1,754.9) |
| TBil (µmol/liter)          | ND              | 20.9 (15.0–125.7)   | 264.0 (177.5–532.6) |
| ALB (g/liter)              | ND              | 41.5 (35.7–47.3)    | 30.5 (22.1–39.8)   |
| AFP                        | ND              | 10.6 (2.7–154.1)    | 78.2 (21.2–385.6)  |
| Cr (µmol/liter)            | ND              | 75.0 (52.4–95.4)    | 71.0 (40.0–97.3)   |
| INR                        | ND              | 1.0 (0.9–1.1)       | 1.6 (1.0–3.2)      |
| MELD score                 | ND              | 7.0 (3.6–11.2)      | 20.6 (13.6–25.8)   |

<sup>a</sup> Abbreviations: ND, not determined; NC, normal controls; CHB, chronic hepatitis B; ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; TBil, total bilirubin; ALB, albumin; Cr, creatinine; INR, international normalized ratio; MELD, model for end-stage liver disease.

<sup>b</sup> Except as indicated, data are shown as the median (10th to 90th percentile).

<sup>c</sup> Data were analyzed with the Kruskal-Wallis H test and Mann-Whitney nonparametric U test. Bold indicates a P value of <0.05.
Quanzhou (no. 20140308), and written informed consent was obtained from each participant.

**ELISA.** The concentrations of TGF-β1, IL-9, IL-10, IL-17, IL-22, IL-23, IL-31, IL-33, and IL-35 in plasma were determined by enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer’s instructions (Market Inc., San Jose, CA, USA). The data were read at 450 nm in a microplate reader (ELx800; BioTek Instruments, Inc., Winooski, VT, USA).

**Assessment of other clinical parameters.** Serum albumin (ALB), alanine aminotransferase (ALT), TBil, creatinine, and other biochemical indices were determined with an automatic biochemical analyzer (LX-20; Beckman, USA). The PT and INR were measured with an automated coagulation analyzer (IL TOP700; Werfen Group, San Jose, CA, USA). HBsAg, anti-HBs, hepatitis B e antigen (HBeAg), anti-HBe, total IgM anti-HBc, anti-HCV, anti-HDV, HIV, and alpha-fetoprotein (AFP) were all measured by the Architect QT assay (i2000SR; Abbott, USA). Serum HBV DNA was determined using a commercial real-time PCR kit in a PE 9700 thermal cycler (Perkin-Elmer, Boston, MA, USA) according to the manufacturer’s instructions. The detection limit for HBV DNA was $1 \times 10^4$ copies $\cdot$ ml$^{-1}$.

**Statistical analysis.** All data were analyzed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as the median (10th to 90th percentile) unless specified. The Kruskal-Wallis H test, Mann-Whitney nonparametric U test, and chi-square test were used to analyze significant differences. The Wilcoxon signed-rank test was used for paired comparisons. Spearman’s rank correlation was performed between variables. A correlation matrix analysis and a cluster tree of cytokines were performed to determine the interrelationship among 9 cytokines. A two-sided $P$ value of $<0.05$ was considered a significant difference.

**RESULTS**

**Clinical data from the three groups.** Clinical data from the NC, CHB, and ACLF groups are shown in Table 1. There were more male patients in the ACLF group than in the NC and CHB groups ($P = 0.005$). TBil levels, AFP levels, and INR and MELD scores were clearly increased in the ACLF group compared to the CHB groups ($P < 0.001$ for both), while ALB levels were lower in the ACLF group than in the CHB group. The age, the positive rate for HBeAg, mean HBV DNA loads, and Cr levels were similar in the two groups.

**Serum levels of components of the TGF-β1/IL-31 pathway are increased in ACLF patients.** The comparisons of serum cytokine levels in the NC, CHB, and ACLF groups are shown in Fig. 1. The levels of TGF-β1 and IL-31 were highest in the ACLF group (440.3 [279.6 to 2,297.7] pg/ml and 30.2 [19.6 to 102.5] pg/ml, respectively) compared with the CHB group (77.7 [66.5 to 282.3] pg/ml and 7.3 [3.2 to 28.0] pg/ml, respectively; $P < 0.001$ for both) and the NC group (72.6 [46.5 to 123.4] pg/ml and 3.9 [0.9 to 10.3] pg/ml, respectively; $P < 0.001$ for both). There were also significant differences between the CHB and NC groups ($P = 0.016$ and 0.004, respectively). IL-17, IL-22, and IL-33 levels were higher in both the CHB and ACLF groups than in the NC group (all $P < 0.01$), but there was no significant difference between the CHB and ACLF groups. The ACLF group had higher levels of IL-35 than the NC group ($P = 0.044$), although some of the differences were not statistically significant. The levels of IL-9, IL-10, and IL-23 were similar in all three groups (all $P > 0.05$).
The patients with CHB and ACLF were further divided into HBeAg-positive \((n/11005 = 21)\) and HBeAg-negative \((n/11005 = 14)\) groups. We found that there was no statistically significant difference in the serum cytokine levels between the two groups. Correlation between activation of the TGF-β/IL-31 pathway and the extent of liver injury and disease severity. Elevation of ALT usually indicates liver cell damage, while total TBil and ALB are clinical indices reflecting the extent of liver injury and liver function decompensation. AFP is a glycoprotein most commonly found in HCC patients and also exists in serum in pregnancy, active liver disease, and embryonic gonad tumors (26). We excluded pregnancy, HCC, and embryonic gonad tumors, so AFP was related to active hepatic injury in our enrolled subjects. The TGF-β1 and IL-31 \((n/11005 = 35)\) levels were negatively correlated with ALB \((r/11005 = 0.717, P < 0.001, 0.727, P < 0.001)\) and were positively correlated with total bilirubin \((b), AFP (c), and Child-Pugh score (f), but they were not correlated with ALT \((d), HBV DNA load (e), and MELD score (g).\)

FIG 2 Increased levels of TGF-β1 and IL-31 are positively correlated with the extent of liver injury and disease severity. Spearman’s rank correlation was performed between variables. TGF-β1 and IL-31 levels were negatively correlated with ALB \((a)\) and were positively correlated with total bilirubin \((b), AFP (c),\) and Child-Pugh score \((f), but they were not correlated with ALT \((d), HBV DNA load (e), and MELD score (g).\)

The Child-Pugh score, which was derived from biochemical indicators, has been used to assess the prognosis of liver failure (LF), the required strength of treatment, and the necessity of liver transplantation (27). The MELD score is a reliable measure of short-term mortality risk in patients with end-stage liver disease etiology and severity (20). There were significant positive correlations between the Child-Pugh score and the serum levels of TGF-β1 \((r/11005 = 0.510; P = 0.031), IL-31 \((r/11005 = 0.563; P = 0.015), and IL-17 \((r/11005 = 0.496; P = 0.036)\) in patients with ACLF, but there were no correlations between the MELD score and TGF-β1 \((r/11005 = 0.284; P = 0.254), IL-31 \((r/11005 = 0.238; P = 0.341), and other cytokines (Fig. 2f and g).\)

Associations between TGF-β1/IL-31 pathway and survival. We also used patient survival as an index of disease severity in the ACLF group. The AFP level \((116.4)\) in the survivors was higher than that in the nonsurvivors \((56.0)\), probably suggesting more active regeneration of new hepatocytes in the livers of survivors, but this was not statistically significant \((Z/11005/11002 = 0.108; P = 0.958).\) The serum levels of TGF-β1 and IL-31 were significantly higher in the nonsurvivor group \((1,302.1 \pm 286.9 to 2,638.8)\ pg/ml and 58.4 \pm 15.3 to 123.6) pg/ml, respectively) than in the survival group \((379.1 \pm 277.6 to 776.2) pg/ml \[(P = 0.013)\) and 25.0 \pm 20.4 to 37.7) pg/ml \[(P = 0.013), respectively)\). A poor prognosis was also associated with high levels of IL-9 \((P = 0.013), IL-10 \((P = 0.013), IL-23 \((P = 0.016), and IL-35 \((P = 0.06)\) (Fig. 3). Among them, TGF-β1, IL-31, IL-9, and IL-10 showed the same highest values...
for the area under the concentration-time curve (AUC) (0.875; \( P = 0.013 \)). However, only IL-31 showed the highest sensitivity and specificity in predicting nonsurvival within the ACLF patients (85.7% and 100.0% at the cutoff value of 39.27, respectively). Furthermore, IL-23 and IL-35 also displayed high sensitivity and specificity in predicting nonsurvival among the patients with ACLF (Table 2).

**Associations between the TGF-β1/IL-31 pathway and other cytokines.** The serum TGF-β1 levels were strongly correlated with the levels of IL-31 (r = 0.947; \( P < 0.001 \)) in all subjects (n = 53), and they were all correlated with IL-17 (r = 0.442 and 0.465, respectively; \( P < 0.01 \) for both), IL-22 (r = 0.470 and 0.582, respectively; \( P < 0.001 \) for both), and IL-33 (r = 0.417 and 0.448, respectively; \( P < 0.01 \) for both), but not with IL-9 (r = 0.157 and 0.122, respectively; \( P > 0.05 \) for both), IL-10 (r = 0.130 and 0.099, respectively; \( P > 0.05 \) for both), IL-23 (r = 0.220 and 0.186, respectively; \( P > 0.05 \) for both), or IL-35 (r = 0.231 and 0.213, respectively; \( P > 0.05 \) for both), in CHB and ACLF patients (n = 35). (Fig. 4). We also performed correlation matrix and cluster tree analyses of interrelationships among all 9 cytokines. We found that there seemed to be relationships between TGF-β1, IL-9, IL-10, IL-17, IL-22, IL-23, IL-31, IL-33, and IL-35. There were two main clusters formed on TGF-β1, suggesting a central position of TGF-β1 in interregulation of those cytokines. We also observed that TGF-β1 and IL-31, IL-17 and IL-22, and IL-9, IL-10, IL-23, IL-33, and IL-35 were grouped together. (Fig. 4e and f).

**Changes in the TGF-β1/IL-31 pathway after nucleos(t)ide analog antiviral treatment.** The follow-up data for 12 CHB patients showed that the levels of TGF-β1, IL-31, IL-17, IL-22, and IL-35 were significantly decreased compared with those pretreatment (all \( P < 0.05 \)), and the IL-23 and IL-35 levels were slightly decreased (\( P = 0.099 \) and 0.367, respectively). This was in comparison with the IL-9 and IL-10 levels, which were increased (\( P = 0.028 \) and 0.050, respectively) (Fig. 5).

**DISCUSSION**

TGF-β1 is thought to be an upstream molecule of the IL-31–JAK-STAT signal pathway and stimulates the production of IL-31 (19).
Evidence has demonstrated that the TGF-β1/IL-31 pathway is involved in the progression of bleomycin-induced pulmonary fibrosis in mice (18, 19). In the present study, we found for the first time that the TGF-β1/IL-31 pathway may be involved in the progression of liver injury in ACLF and was correlated with the extent of liver injury and survival, as well as being associated with recovery in CHB. More important, the TGF-β1/IL-31 pathway showed the highest sensitivity and specificity in predicting nonsurvival in ACLF patients. Although this study is descriptive in nature, the results suggest active involvement of the described cytokines in the hepatic inflammatory process, which we consider to be both novel and interesting. Our observations may contribute to a better understanding of the relevant cytokines involved in the pathogenesis of liver injury and inflammation and may lead to further investigation into the mechanisms of action of these cytokines as well as into other viral and nonviral causes of liver injury.

Currently, the pathogenesis of liver injury in ACLF remains unclear, but the “three-beat” hypothesis (28), that is, immunoenhancement, hypoxic ischemia, and endotoxemia, may explain the progressive liver injury and malfunction of this organ. Previous studies have focused on the first “beat,” and they suggest that natural killer (NK) cells (29), Kupffer cells (30), dendritic cells (DCs) (24), monocytes, Th1 cells (30), Th17 cells (23, 31), regulatory T (Treg) cells (21), and other immunologically relevant cells are all involved in the pathogenesis of liver injury in ACLF. The increased Treg cells, monocytes, and other cells lead to elevated TGF-β1 expression. TGF-β1 can induce Smad2 phosphorylation and then activates the binding of Smad3 to IL-31 promoters, before finally stimulating the production of IL-31 (18). TGF-β1 also delays liver regeneration and promotes perisinusoidal fibrosis and hepatocyte apoptosis (5), while IL-31 promotes inflammation responses (6). Therefore, we hypothesize that both are the essential players of the same signal pathway, which could further trigger the activity of downstream players, leading to hepatic injury. Such an understanding is supported by the markedly elevated serum levels of TGF-β1 and IL-31 in ACLF patients and by the strong correlation between them in our study. Furthermore, we also found the serum levels of IL-17, IL-22, IL-33, and IL-35 were significantly increased in ACLF patients compared to the NC group, and serum levels of TGF-β1 and IL-31 were all positively correlated with IL-17, IL-22, and IL-33, which have all been demonstrated to be proinflammatory cytokines in HBV-related diseases (23, 32, 33). Also, we found that serum levels of IL-9, IL-10, and IL-35 were elevated in the nonsurvivor ACLF patients. These cytokines are generally considered to be anti-inflammatory or cryoprotective. Elevated cryoprotective cytokines probably represent the effort to balance proinflammatory cytokines which are circulating at overwhelming levels upon severe liver injury. We reason that production and release of the cryoprotective cytokines is proportional to the proinflammatory cytokines, which were higher in the nonsurvivors than the survivors. Our findings suggest that the TGF-β1/IL31 pathway may upregulate expression levels of other inflammatory cytokines and could facilitate the progressive course of liver injury in ACLF. Therefore, the TGF-β1/IL-31 pathway may be involved in liver injury by direct inflammatory function and by modulating the expression of other inflammatory cytokines of the innate and adaptive immune cells. We are not sure whether in this study increased expression of TGF-β1 and IL-31 initially triggered the liver injury in ACLF or was just a part of the inflammatory reaction to the liver injury, but we are certain that high levels of expression of TGF-β1 and IL-31 as well as other inflammatory cytokines would precipitate and worsen the pathological process of the liver injury, which in turn could facilitate expression of more of those cytokines (28). For instance, IL-21 is an important inflammatory factor and was possibly involved in the liver injury by regulating the function of innate and adaptive immunocompetent cells and/or affecting the expression of other inflammatory cytokines (28). Such a cyclic process could expand liver injury and eventually cause massive cell death, resulting in the failure of liver function. The results suggest that modulation of the TGF-β1/IL-31 pathway may potentially regulate the immunological process of ACLF and yield a beneficial outcome as a part of immunotherapy.

The severity of ACLF can be influenced by a number of factors, including age, HBeAg status, HBV DNA load, and the presence of underlying cirrhosis (20, 22). Other factors might influence prognosis, such as sepsis, diabetes, and concurrent kidney disease. In our view, the severity of ACLF is directly determined by the extent of liver injury. The more extensive the liver injury, the more severe the ACLF. We would expect to see strong correlations between the markers reflecting the extent of liver injury and the severity or survival of ACLF. In our study, we found that TGF-β1 and IL-23 were negatively correlated with ALB and positively correlated with TBil and AFP but were not correlated with ALT, suggesting that TGF-β1 and IL-23 may reflect the extent of liver injury, rather than inflammation. Furthermore, we also found clear correlations

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**TABLE 2 Predictive values of TGF-β1 and IL-31 for nonsurvival of ACLF patients**

| Cytokine | AUC (µg · h/ml) | 95% confidence interval | P value a | Sensitivity (%) b | Specificity (%) b | Cutoff value (pg/ml) |
|----------|-----------------|------------------------|-----------|-------------------|-------------------|---------------------|
| IL-31    | 0.857           | 0.598–1.116            | 0.013     | 85.7              | 100.0             | 39.27               |
| TGF-β1   | 0.857           | 0.638–1.077            | 0.013     | 85.7              | 81.8              | 449.12              |
| IL-9     | 0.857           | 0.680–1.034            | 0.013     | 85.7              | 72.7              | 53.18               |
| IL-10    | 0.857           | 0.681–1.034            | 0.013     | 71.4              | 72.7              | 19.88               |
| IL-23    | 0.844           | 0.649–1.039            | 0.016     | 85.7              | 81.8              | 1,340.57            |
| IL-35    | 0.844           | 0.655–1.033            | 0.016     | 85.7              | 72.7              | 88.92               |
| IL-33    | 0.747           | 0.506–0.988            | 0.085     | 85.7              | 54.5              | 9.83                |
| IL-22    | 0.494           | 0.214–0.773            | 0.964     | 57.1              | 63.6              | 116.74              |
| IL-17    | 0.455           | 0.154–0.755            | 0.751     | 57.1              | 36.4              | 39.41               |

a Bold indicates a P value of <0.05.
b Levels of TGF-β1, IL-31, IL-9, IL-10, IL-23, and IL-35 displayed high sensitivity and specificity in predicting nonsurvival among the patients with ACLF, but IL-31 showed the highest predictive value.
between TGF-β1/IL-31, the Child-Pugh score, and survival. IL-31 particularly displayed the highest sensitivity and specificity (85.7% and 100.0%, respectively) in predicting nonsurvival within the ACLF patients, indicating that TGF-β1/IL-31 may be used as a potential marker of disease severity. Meanwhile, the follow-up data showed that the levels of TGF-β1, IL-31, and their associated inflammatory cytokines, such as IL-22, IL-17, and IL-33, were decreased with the recovery of CHB patients, who were treated with nucleos(t)ide analogs. Our findings are consistent with published studies that showed that cytokines can also influence disease progression (4, 23, 30, 32).

Surprisingly, there were no significant associations between the TGF-β1/IL-31 pathway and MELD scores in ACLF patients, which was similar to the results of a study by Hu et al. (28), which showed that the frequency of IL-21-producing CD4 T cells was not associated with MELD scores in ACLF patients. Possible explanations for the absence of correlation were that the enrolled ACLF patients were still in the early stage of LF, and the levels of TBil, Cr, and INR, the indices of calculated MELD scores, were not yet at their highest, or we may speculate that the metabolism of TGF-β1 and IL-31 may be blocked because of liver dysfunction.

There are some limitations to our study. First, we were unable to obtain liver biopsy specimens from ACLF patients because of their very fragile status, so we did not know the differences in the TGF-β1/IL-31 pathway between liver immune environment and peripheral blood immune responses. Second, only 12 CHB patients were followed up, and samples at two time points were obtained. The other 5 CHB patients and ACLF survivors were lost during the follow-up period for various reasons. Finally, owing to the limited blood sample, we could not determine the expression of TGF-β1 and IL-31 in CD4 T cells. Therefore, we will deter-

**FIG 4** Relationship between the TGF-β1/IL-31 pathway and other cytokines and cluster tree of cytokines. Spearman’s rank correlation was performed between variables. (a to d) TGF-β1 levels were strongly correlated with IL-31 levels (a), and they were all positively correlated with IL-17 (b), IL-22 (c), and IL-33 (d). (e) Interrelationships among all 9 cytokines. (f) A hierarchical cluster analysis shows that TGF-β1 and IL-31 were grouped together.
mine the frequencies of TGF-β1-secreting CD4+ T cells and IL-31-secreting CD4+ T cells in our future research.

In conclusion, our study showed that serum TGF-β1 levels were strongly correlated with IL-31, and they were all elevated significantly in ACLF patients and correlated with the extent of liver injury, Child-Pugh scores, and survival. The TGF-β1 and IL-31 levels were also increased significantly in CHB patients, and the reduction in the expression level was associated with liver injury recovery. More importantly, TGF-β1 and especially IL-31 showed not only the highest AUC value but also the highest sensitivity and specificity in predicting prognosis and disease progression of ACLF.

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There is no conflict of interest.

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