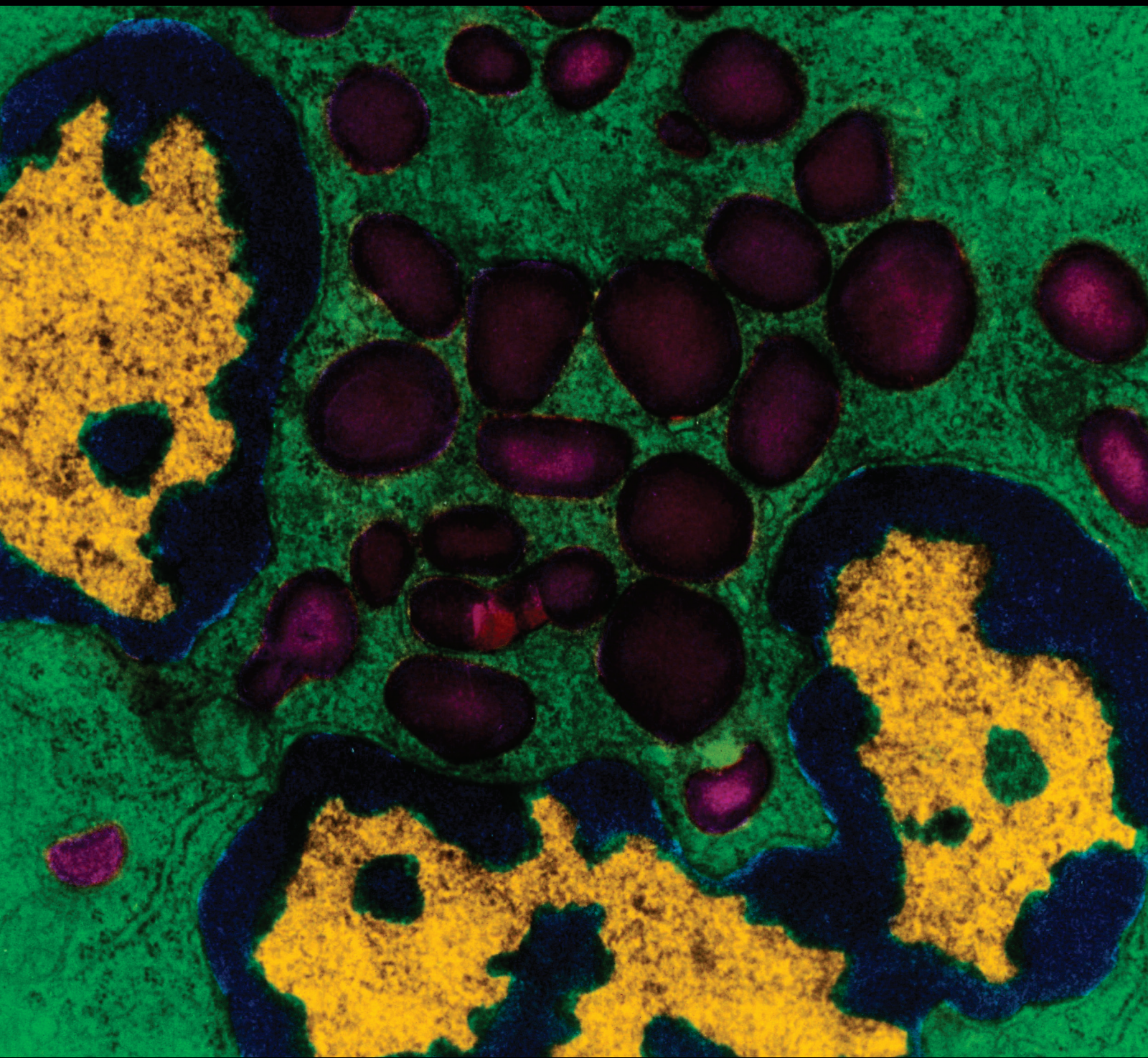
Mediators of Inflammation

Inflammation in Liver Diseases

Lead Guest Editor: Dechun Feng
Guest Editors: Partha Mukhopadhyay, Ju Qiu, and Hua Wang



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Editorial Board

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Editorial

Inflammation in Liver Diseases

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The liver is the largest solid organ in our body responsible for nutrient metabolism and protein synthesis. Recently, accumulating evidence showed that the liver was not only a major metabolic organ but also an "immunologic organ." Due to a strong and specific blood supply route through the liver, it maintains a unique immune microenvironment. Many liver-resident antigen-presenting cells also modulate immune regulatory function. Liver-resident Kupffer cells have an important role in phagocytosis to prevent invasion of pathogenic organisms from the intestine. Innate lymphocytes, including both natural killer cells and natural killer T cells, are enriched in the liver. The liver is also the major organ to produce acute-phase proteins which are closely associated inflammatory reactions.

The dysregulation of immune cells in the liver was critical in the pathogenesis of almost all types of liver diseases including viral hepatitis, autoimmune hepatitis, fatty liver, alcoholic liver diseases, cirrhosis, and drug/toxin-induced injury. The studies on liver inflammation will greatly improve the understanding of the mechanism of how liver immune cells interacted with hepatocytes and other cells in the liver to cause liver damage as well as liver repair after damage. These studies will also be very helpful in the development of a novel effective treatment for various liver diseases in the clinical setting.

In this special issue, we have invited a few papers that address such issues.

The first paper of this issue analyzed the change of etiology of liver cirrhosis patients in a hospital from 2002 to 2013 in China. They showed that the top four etiologies of cirrhosis were HBV, HCV, ALD, and autoimmune liver disease. The prevalence of HBV cirrhosis has decreased in the recent 12 years, which is related with the increased coverage of HBV vaccination in China. In contrast, alcoholic cirrhosis has increased significantly. The study supported the success of HBV vaccination in reducing HBV-related end-stage liver diseases and also suggested more attention should paid to alcoholic liver disease.

The second paper of this issue reviewed the role of IL-33, an IL-1 cytokine family member, in the pathogenesis of liver diseases. IL-33 functions as an alarmin that is released when barriers are breached. IL-33 binds to its receptor ST2 and activates NF-κB, ERK, and JNK signaling. IL-33 is upregulated in fatty liver, and the treatment of IL-33 alleviated hepatic steatosis. In hepatitis, the role of IL-33 remains controversial. Most studies support IL-33 as protective in hepatitis; however, one report showed that the treatment of IL-33 exacerbated Con A-induced hepatitis. In addition, IL-33 is considered as a cytokine that can promote liver fibrosis in several animal models, and the levels of IL-33 are positively correlated with the severity of fibrosis in patients.

The third paper of this issue analyzed the prognostic value and causes of pretreatment liver injury in de novo diffuse large B-cell lymphoma (DLBCL) patients. Multivariate

analysis revealed that liver dysfunction, advanced Ann Arbor stage, and elevated lactate dehydrogenase (LDH) were independent adverse prognostic factors of both progression-free survival (PFS) and overall survival (OS).

The fourth paper reviewed novel and important signaling molecules involved in the process of liver regeneration. The authors summarized cytokines in the priming phase; growth factors, Wnt signaling, and exosomes in the proliferation phase; and TGF-β/TGF-β family in the termination of liver regeneration. These information will be very helpful in understanding the mechanisms of liver regeneration and promoting liver repair after injury.

The fifth paper studied the protective effects of magnesium isoglycyrrhizinate (MgIG) in treating alcoholic liver disease. The authors used a novel chronic plus binge ethanol feeding-induced liver injury to evaluate the therapeutic effects of MgIG. MgIG significantly reduced the elevation of liver enzymes caused by alcohol and hepatic steatosis. The hepatoprotective effect of MgIG was associated with suppression of neutrophil ROS production as well as hepatocellular oxidative stress.

The sixth paper identified a novel biomarker cytokeratin 18 epitope M30 (M30 CK-18), which was correlated with liver inflammatory activity. M30 CK-18 can also discriminate patients with mild versus moderate-advanced fibrosis. The authors found that M30 CK-18 serum concentration had good sensitivity and specificity in discriminating mild versus moderate/severe fibrosis and inflammation even in patients with normal ALT activity.

The seventh paper investigated the role of hypoxia-inducible factor (HIF)1α in macrophage polarization. They found that overexpression of HIF1α in myeloid cells were in hyperinflammatory state characterized by the upregulation of M1 markers. Further metabolomics studies showed that HIF1α overexpression led to the increased glycolysis and pentose phosphate pathway intermediates which facilitated the M1 phenotype shift in macrophages.

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Research Article

HIF1α-Induced Glycolysis Metabolism Is Essential to the Activation of Inflammatory Macrophages

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Hypoxia-inducible factor (HIF) 1α is a metabolic regulator that plays an important role in immunologic responses. Previous studies have demonstrated that HIF1α participates in the M1 polarization of macrophages. To clarify the mechanism of HIF1α-induced polarization of M1 macrophage, myeloid-specific HIF1α overexpression (Lysm HIF1α lsl) mice were employed and the bone marrow-derived and peritoneal macrophages were isolated. RT-PCR results revealed that HIF1α overexpression macrophage had a hyperinflammatory state characterized by the upregulation of M1 markers. Cellular bioenergetics analysis showed lower cellular oxygen consumption rates in the Lysm HIF1α lsl mice. Metabolomics studies showed that HIF1α overexpression led to increased glycolysis and pentose phosphate pathway intermediates. Further results revealed that macrophage M1 polarization, induced by HIF1α overexpression, was via upregulating the mRNA expression of the genes related to the glycolysis metabolism. Our results indicate that HIF1α promoted macrophage glycolysis metabolism, which induced M1 polarization in mice.

1. Introduction

Macrophages are the main component of innate immunity and play important roles in various inflammatory diseases, including hepatitis, vascular diseases, inflammatory bowel diseases, rheumatoid arthritis, and airway inflammation [1–5]. Activated macrophages are commonly divided into two polarized phenotypes, classically activated M1 and alternatively activated M2. Macrophages activated by interferon γ or toll-like receptor agonists polarize to the M1 phenotype [6], which are proinflammatory macrophages and play a central role in the host's defense against infection and inflammatory diseases [7, 8]. Macrophages activated by Th2 cytokines, IL-4, and IL-13 are polarized to M2 phenotype, which are associated with inflammation relief and tissue remodeling [9, 10]. Macrophage activation can be altered by disrupting cellular energy metabolism [11, 12]. Recent studies have demonstrated that M1 macrophages demand glycolysis, while M2 macrophages require fatty acid oxidation [13, 14]. However, the metabolomics profiling and the metabolic mechanism of macrophages polarization remained undefined.

Hypoxia-inducible factor 1 (HIF1) has emerged as one of the central regulators of inflammation mediated by myeloid cells [15, 16]. HIF1 is an α and β heterodimer [15, 17]. Whereas HIF1β is constitutively expressed in cells regardless of O_2 tension [18], HIF1α protein increases exponentially in response to reduced O_2 concentration [19]. HIF1 has displayed a significant role in regulating cellular ATP concentration and myeloid cell function including cell aggregation, motility, invasiveness, and bacterial killing [20–22]. Importantly, it has been reported that HIF1 participates in the regulation of macrophage polarization [20]. As glucose metabolism determines polarization of macrophages [23, 24], whether glucose metabolism is involved in HIF1α-induced macrophage polarization process has remained unclear.

2. Materials and Methods

2.1. Chemicals and Reagents. RPMI 1640 medium was purchased from Gibco. Fetal Bovine Serum (FBS), penicillin, and streptomycin were purchased from HyClone. GM-CSF was purchased from PeproTech. Ammonium acetate, LPS, oligomycin, carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), rotenone, and antimycin A were purchased from Sigma. BBL™ Thioglycollate Medium was purchased from BD Biosciences, US. [5-13C]glutamine was obtained from Cambridge Isotope Laboratories. HPLC grade ammonium hydroxide, acetonitrile, and methanol were purchased from Fisher Scientific. Deionized water was produced by a Milli-Q system.

2.2. Animals. Lsl-HIF1 dPA mice were obtained as described previously [25]. For myeloid-specific HIF1α overexpression, Lsl-HIF1 dPA mice were crossed with mice harboring the Cre recombinase under control of the lysozyme M (Lysm) promoter, which is found only in myeloid lineage cells, to obtain the Lysm HIF1α lsl mice. The wild-type (WT) and Lysm HIF1α lsl mice were littermate and on a C57BL/6 J background, after backcrossing with C57BL/6J mice for over ten generations. All the animal protocols were approved by the Animal Care and Use Committee of Peking University.

2.3. Peritoneal Macrophage. WT and Lysm HIF1α lsl mice (6- to 8-weeks old) were injected intraperitoneally with 4% thioglycollate solution (2 ml). Three days later, peritoneal cells were harvested by injecting the peritoneal cavity with PBS containing 10% FBS. Primary peritoneal macrophages were cultured with RPMI-1640 medium supplemented with 10% FBS. Medium was changed 2–4 h later. Thioglycollate-elicited peritoneal macrophages were attached on plates and continued culturing for 6 to 24 h.

2.4. Bone Marrow-Derived Macrophages (BMDMs). Bone marrow cells were collected from WT and Lysm HIF1α lsl mice (4- to 6-weeks old). Adherent macrophages were cultured for 3 days in RPMI-1640 supplemented with 10% FBS and GM-CSF (10 ng/mL). Then, the medium was changed and the attached macrophages were obtained after another 3 days. To obtain the M1 polarization, macrophages were continued culturing for 2 days in RPMI-1640 supplemented with 10% FBS and LPS (10 ng/mL).

2.5. Quantitative RT-PCR. Total RNA was isolated from peritoneal macrophages or BMDMs using TRIzol reagent. cDNA was obtained using the M-MLV reverse transcriptase kit according to the manufacturer's instructions. RT-PCR amplification was performed using an Mx3000 Multiplex Quantitative PCR System and SYBR Green I reagent. Gene expression levels were normalized to the internal control 18S rRNA.

2.6. Extracellular Flux Analysis. An XF24 Extracellular Flux Analyzer was used to measure the respiratory conditions of murine peritoneal macrophages. Cells were plated at 5×10^4 cells/well in 24-well XF microplates and cultured for 6 h. RPMI-1640 medium was replaced with XF base medium supplemented with 25 mM glucose and 2 mM pyruvate. After 1 h of incubation in a CO_2-free incubator at 37°C, the oxygen consumption rate (OCR) and extracellular acidification rates (ECAR) were measured following the manufacturer's instruction. Mitochondrial stress tests were performed under basal conditions or with the treatment of metabolic reagents, including 1 mM oligomycin, 1 mM FCCP, 1 mM rotenone, and 1 mM antimycin A. ECAR was calculated by Wave software.

2.7. Metabolomics Analysis. Analysis of metabolites was performed with a liquid chromatography-tandem mass spectrometry. For metabolite extraction, cultured cells were washed with saline twice, lysed in 80% aqueous methanol (v/v), and equilibrated at −80°C for 20 min. [5-13C]glutamine was added as an internal standard. Cells were oscillated for 10 min and centrifuged with the speed of 14,000g for 10 min at 4°C. Cell supernatants of metabolite extracts were collected, dried, and stored at −80°C before injection.

For liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, samples were reconstituted in water and analyzed using a QTRAP 5500 LC-MS/MS system (AB SCIEX) coupled with an ACQUITY UHPLC System (Waters Corporation). An Xbridge Amide column (100 × 4.6 mm i.d., 3.5 Lm; Waters Corporation) was employed for compound separation at 30°C. The mobile phase A was 5 mM ammonium acetate in water with 5% acetonitrile, and mobile phase B was acetonitrile. The linear gradient used was as follows: 0 min, 90% B; 1.5 min, 85% B; 5.5 min, 35% B; 10 min, 35% B; 10.5 min, 35% B; 14.5 min, 35% B; 15 min, 85% B; and 20 min, 85% B. The flow rate was 0.5 ml/min. MultiQuant v3.0 software (AB SCIEX) was used to process all raw liquid chromatography-mass spectrometry data and integrate chromatographic peaks. Integrated peak areas corresponding to metabolite concentrations were further analyzed using the MetaboAnalyst website (http://www.metaboanalyst.ca). Metabolite abundance was expressed relative to the internal standard.

2.8. Statistical Analysis. All data are presented as the mean ± SEM. Comparisons of data sets were performed using unpaired Student's t-tests for comparing two groups. Statistical analyses were performed using GraphPad Prism (GraphPad Software). A P value at $^*P < 0.05$ and $^{**}P < 0.01$ was considered statistically significant for all experiments.

3. Results

3.1. HIF1α Induced M1 Polarization of Macrophages. In this study, we used Lysm HIF1α lsl mice and WT mice to testify whether HIF1α in macrophages affects macrophage polarization as previously reported [20]. The mRNA level of *Hif1α* in bone marrow-derived macrophages (BMDMs) and peritoneal macrophages was confirmed by RT-PCR, displaying approximately threefolds of *Hif1α* expression compared with the WT mice (Figure 1(a)). Then, we examined the relative mRNA levels of M1 and M2 markers in peritoneal macrophages and BMDMs. The mRNA expressions of M1 markers,

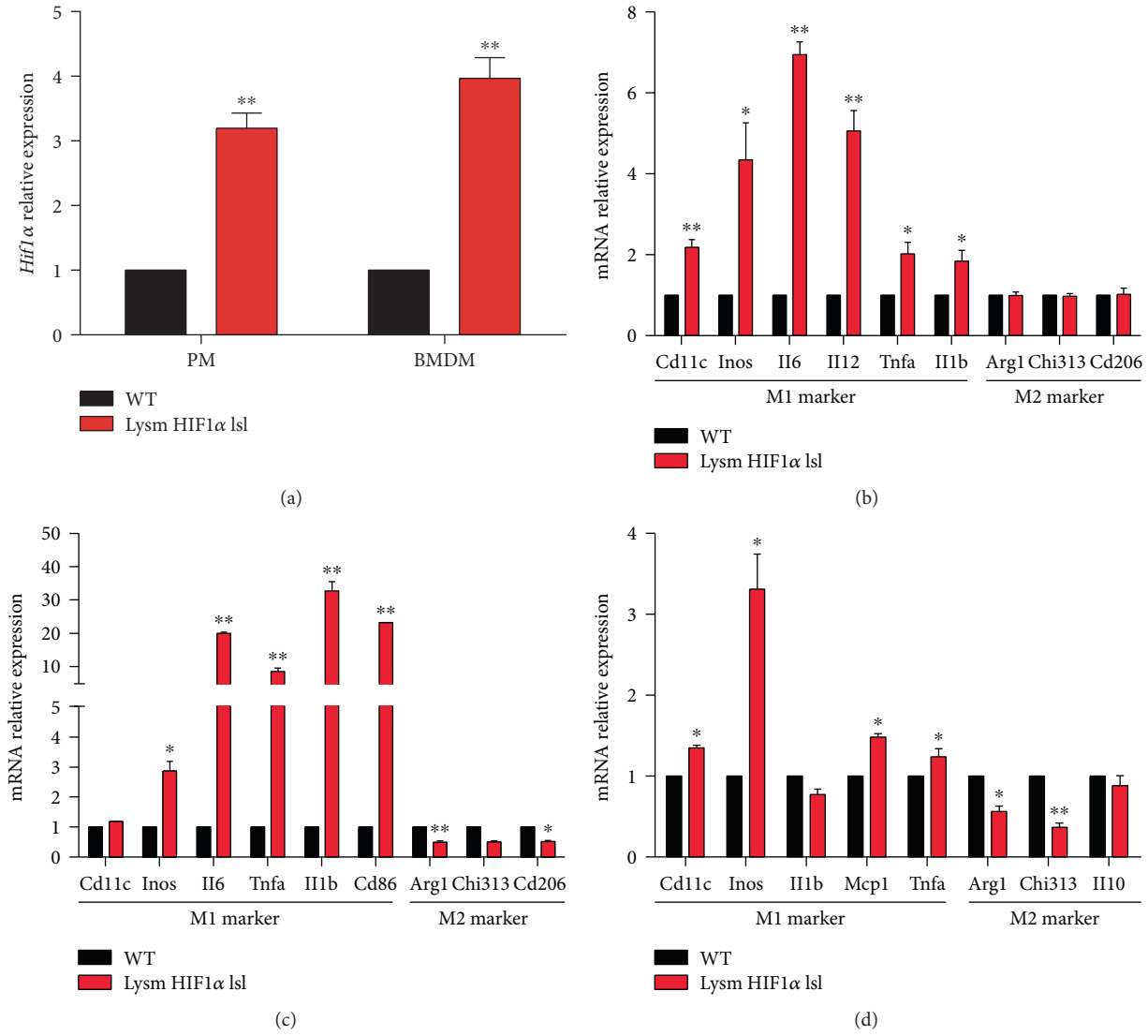


Figure 1: HIF1α induced M1 polarization of macrophages. (a) The relative mRNA level of HIF1α in the BMDMs and peritoneal macrophages of WT mice and Lysm Hif1α lsl mice. (b) The relative mRNA levels of M1 and M2 markers in the peritoneal macrophages isolated from WT mice and Lysm HIF1α lsl mice for 6 h. (c) The relative mRNA levels of M1 and M2 markers in the peritoneal macrophages isolated from WT mice and Lysm HIF1α lsl mice for 24 h. (d) The relative mRNA levels of M1 and M2 markers in the BMDMs isolated from the WT mice and Lysm HIF1α lsl mice with the treatment of LPS for 48 h. For each gene, mRNA level was normalized to the level of 18S rRNA expression. Statistical comparisons were made using two-tailed Student's t-test (a, b, c, and d). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with WT mice. All values were presented as mean ± SEM for $n = 3$–5 independent experiments in each group.

including *Il6*, *Il1b*, *Inos*, *Tnfα*, and *Cd11c*, were markedly higher in peritoneal macrophages isolated from Lysm HIF1α lsl mice, while the expressions of M2 markers, *Arg1*, *Cd206*, and *Chi313*, showed little difference or even lower compared with WT mice at 6 h (Figure 1(b)) and 24 h (Figure 1(c)). In BMDMs, M1 markers were highly expressed in Lysm HIF1α lsl mice, and the M2 markers were markedly less at the same level by comparison (Figure 1(d)). These results indicate that macrophage HIF1α overexpression induces M1 polarization of macrophages.

3.2. HIF1α Decreased Mitochondrial Oxidation and Promoted Glycolysis Metabolism in Macrophages. Recent studies have indicated that the activation of macrophage polarization was marked by their metabolic programs [23, 24]. Therefore, mitochondrial oxidation was detected in peritoneal macrophages isolated from WT mice and Lysm HIF1α lsl mice. HIF1α overexpressed macrophages displayed a marked lower mitochondrial oxygen consumption rate (OCR) (Figure 2(a)) but a higher extracellular acidification rates (ECAR) (Figure 2(b)), suggesting the promotion of glycolysis metabolism. With the treatment of mitochondrial oxidative inhibitors, including carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), oligomycin, antimycin A, and rotenone, the ratio of mitochondrial oxidation to glycolysis metabolism was decreased in

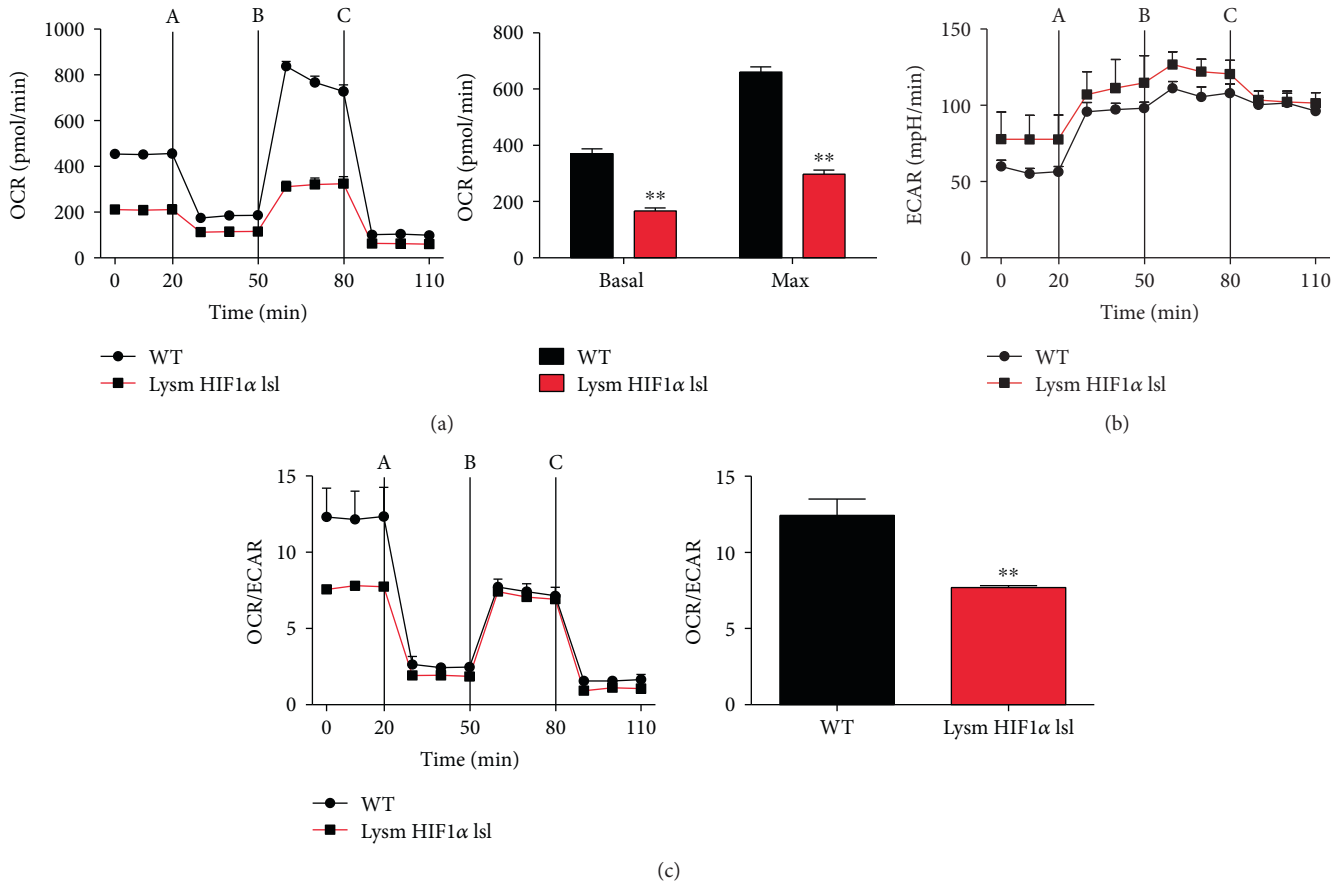
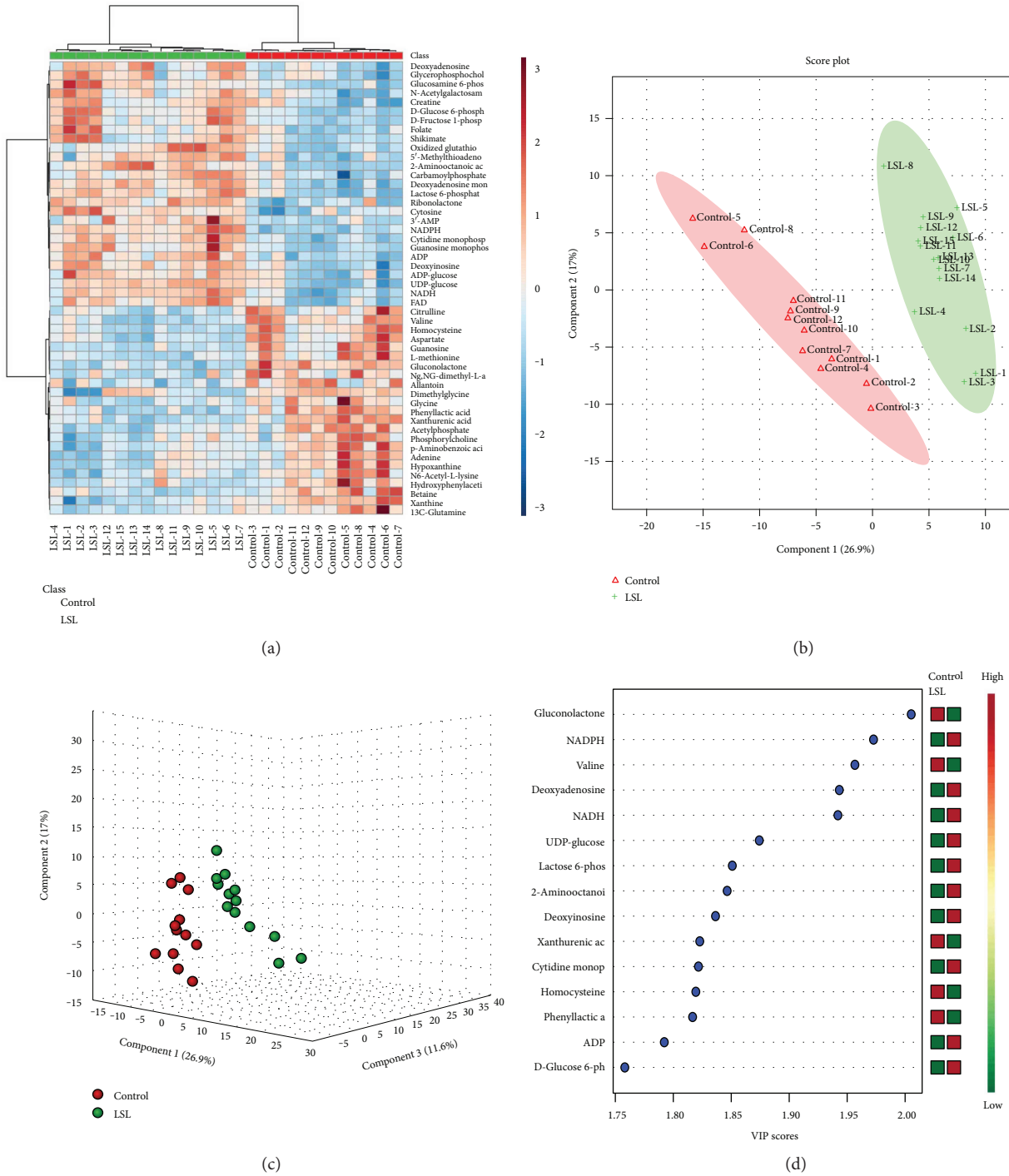


Figure 2: HIF1α decreased mitochondrial oxidation and promotes glycolysis metabolism in macrophages. (a and b) Metabolic respiratory parameters of peritoneal macrophages isolated from the WT mice and Lysm HIF1α lsl mice were measured with the treatment of extracellular flux analyzer: A, oligomycin; B, carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP); C, antimycin A and rotenone. (a) The oxygen consumption rate (OCR) value was measured at the basal level and after the treatment of A, B, and C quantitated on the right panel. Basal OCR was measured before the injection of a, and maximal OCR was calculated by subtracting the nonmitochondrial OCR from the peak OCR after the treatment of B. (b) The extracellular acidification rate (ECAR) value was calculated by the software. (c) The OCR/ECAR ratio was calculated at basal level quantitated on the right panel. Statistical comparisons were made using two-tailed Student's *t*-test (a and c). $^{**}P < 0.01$ compared with WT mice. All values were presented as mean ± SEM for $n = 9$–15 independent experiments in each group.

HIF1α overexpression macrophages (Figure 2(c)). These data indicate that decreased mitochondrial oxidation and increased glycolysis metabolism are induced by HIF1α in macrophages.

3.3. Metabolomics Analysis Showed That HIF1α-Induced Glycolysis Metabolism and Pentose Phosphate Pathway and Decreased Mitochondrial Oxidation. The extracellular flux analysis results revealed the different metabolic mode between macrophages isolated from Lysm HIF1α lsl mice and WT mice. To further explore the detailed changes in metabolic profiling, metabolites were extracted from peritoneal macrophages isolated from Lysm HIF1α lsl mice and WT mice and analyzed using LC-MS/MS. The heatmap generated from hierarchical clustering and a partial least squares discriminant analysis (PLS-DA) plot of metabolites revealed a distinct metabolic profile in macrophages isolated from Lysm HIF1α lsl mice and WT mice (Figures 3(a), 3(b), and 3(c)). VIP scores extracted from the PLS-DA model demonstrated that glycolytic intermediates got relative high VIP scores (Figure 3(d)). Enrichment analysis and pathway analysis showed an apparent disparity in the glycolysis, TCA cycle, and pentose phosphate pathway (Figures 3(e) and 3(f)). Histogram analysis exhibited that the metabolite levels were increased in the glycolysis, including lactate, GADP, G-3-P, 3-PG, 2,3-DPG, FBP, G-6-P, F-6-P, PEP, and BPG (Figure 3(g)), and decreased in mitochondrial oxidation, including fumarate, succinate, citrate, and isocitrate (Figure 3(h)) in Lysm HIF1α lsl mice. Besides, the pentose phosphate pathway, a shunt from the glycolytic pathway, was also activated proved by the increase of d-erythrose-4-phosphate, xylulose-5-phosphate, sedoheptulose-7-phosphate, ribose-5-phosphate, and NADPH levels (Figure 3(i)). The activated pentose phosphate pathway is assumed to provide biosynthetic substrates to support macrophage growth and activation. Thus, metabolomics



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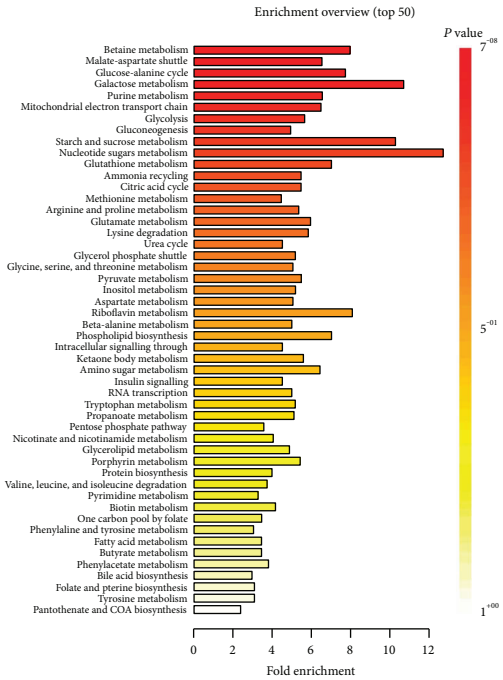

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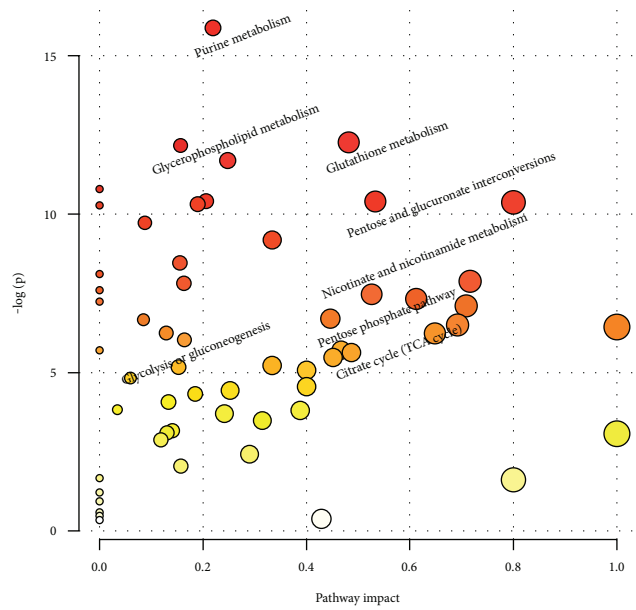

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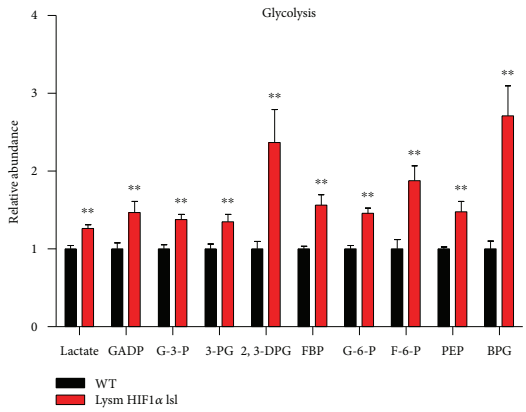

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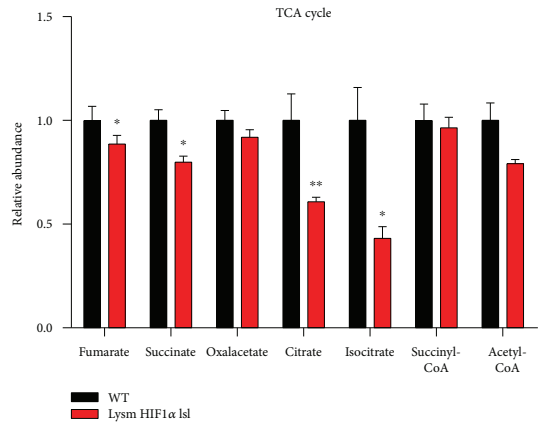

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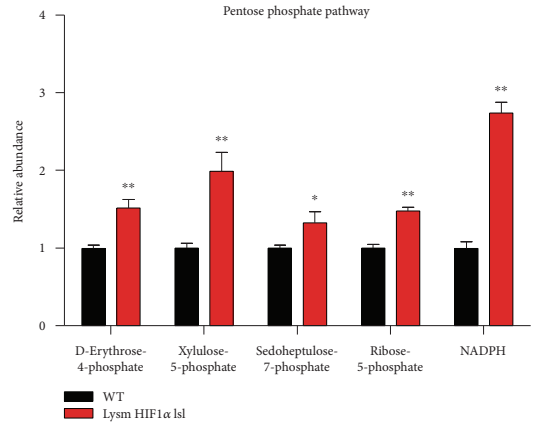

Figure 3: Metabolomics analysis of HIF1α-modified macrophage glycolysis metabolism. (a, b, c, d, e, and f) Peritoneal macrophages were isolated from the WT and Lysm HIF1α lsl mice. An LC-MS/MS system was used to measure the abundance of cellular metabolites. Metabolomics data were analyzed using the MetaboAnalyst website. (a) Heatmap of the intracellular metabolites generated from hierarchical clustering. Red series denoted relative high concentrations and blue series denoted relative low concentrations. (b) 2D PLS-DA score plot. (c) 3D PLS-DA score plot. (d) VIP scores. (e) Overview of metabolite enrichment in HIF1α overexpressed macrophages. (f) Metabolic pathway analysis of HIF1α overexpressed macrophages. (g, h, and i) Relative levels of metabolites in the glycolysis metabolism (g), TCA cycle (h), and pentose phosphate pathways (i). Statistical comparisons were made using two-tailed Student's t-test (g, h, and i). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with WT mice. All values were presented as mean ± SEM for $n = 10$–14 independent experiments in each group. FBP: fructose 1,6-bisphosphate; F-6-P: fructose-6-phosphate; GADP: glyceraldehyde-3-phosphate; G-6-P: glucose-6-phosphate; PEP: phosphoenolpyruvate; 3-PG: 3-phosphoglycerate.

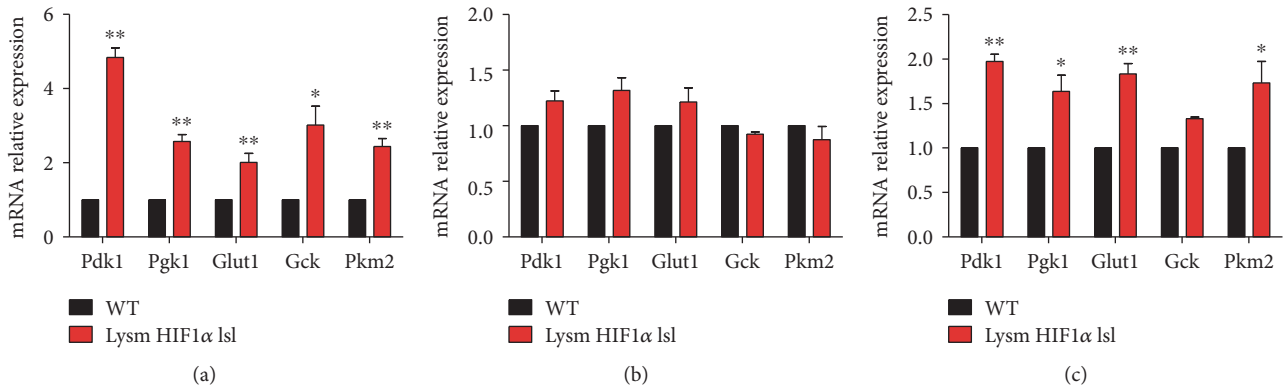

Figure 4: HIF1α activated macrophage glycolysis metabolism-related genes. (a) Relative mRNA levels of *Pdk1*, *Pgk1*, *Glut1*, *Gck*, and *Pkm2* in the peritoneal macrophages isolated from the WT mice and Lysm HIF1α lsl mice for 6 h. (b) Relative mRNA levels of *Pdk1*, *Pgk1*, *Glut1*, *Gck*, and *Pkm2* in the peritoneal macrophages isolated from WT mice and Lysm HIF1α lsl mice for 24 h. (c) Relative mRNA levels of *Pdk1*, *Pgk1*, *Glut1*, *Gck*, and *Pkm2* in the BMDMs isolated from WT mice and Lysm HIF1α lsl mice activated to M1 with the treatment of LPS for 48 h. For each gene, mRNA level was normalized to the level of 18S rRNA expression. Statistical comparisons were made using two-tailed Student's t-test. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with WT mice. All values were presented as mean ± SEM for $n = 3$–5 independent experiments in each group.

analysis showed an enhancement of glycolysis metabolism and pentose phosphate pathway but a decreased mitochondrial oxidation in HIF1α overexpressed macrophages.

3.4. HIF1α-Modified Macrophage Glycolysis Metabolism through Regulation of Glycolytic Gene Expression. The mechanism of the glucose metabolic disparity in HIF1α overexpressed macrophages was explored by analyzing gene expression. mRNA expressions of some glycolytic genes, including *Pdk1*, *Pgk1*, *Glut1*, *Gck*, and *Pkm2*, were higher in peritoneal macrophages isolated from the Lysm HIF1α lsl mice than in the WT mice at both 6 h and 24 h (Figures 4(a) and 4(b)). The similar results were observed in BMDMs isolated from WT mice and Lysm HIF1α lsl mice activated to M1 with the treatment of LPS (10 ng/mL) for 48 h (Figure 4(c)).

4. Discussion

The liver is a site particularly enriched with innate immune cells [26] and the largest metabolic organ in the body that is responsible for various metabolic processes regulating

various functions [27, 28]. Innate immune cells modify and disrupt critical processes implicated in metabolic disease. Meanwhile, metabolic stress initiates a feed-forward cycle of inflammatory responses [29]. Given that HIF1α is a metabolic regulator playing important roles in inflammation [30, 31], we investigated whether the regulation of cellular metabolism by HIF1α controls macrophage polarization and inflammation.

Our study first used HIF1α overexpression mice to validate the previous report that HIF1α promoted the accumulation of M1 macrophages [32–34]. Gene expression profiling of macrophages revealed an increase in markers of M1 macrophages and decreased or unchanged expression of M2 macrophage markers (Figure 1), supporting that HIF1 triggers macrophage polarizing to the M1 phenotype.

Recent findings suggest that cellular metabolism plays an important role during macrophage polarization [23, 35]. Classically activated macrophages secret proinflammatory mediators, accompanied with a shift from mitochondrial oxidation toward glycolysis metabolism [36]. On the contrast, alternatively activated macrophages secrete anti-inflammatory cytokines and declare an increased demand of fatty-acid oxidation [37]. Consistent with these findings, we showed that HIF1α overexpressed macrophages reduced cellular OCR and increased ECAR (Figure 2). The OCR/ECAR ratio was also dramatically decreased, reflecting a preference of glycolysis metabolism compared with mitochondrial oxidation in HIF1α overexpressed macrophages.

Macrophages are capable of coordinating their metabolic programs to adjust their immunological and bioenergetic functional properties. In our study, metabolomics profiling analysis witnessed a splendid disparation of metabolites from peritoneal macrophages isolated from WT mice and Lysm HIF1α lsl mice (Figure 3). Relative concentration of metabolites further demonstrated that HIF1 induced activation of glycolysis metabolism and pentose phosphate pathway and inhibited mitochondrial oxidation in macrophages in Lysm HIF1α lsl mice (Figure 3). Pentose phosphate pathway utilizes glucose to generate NADPH for nucleotide biosynthesis, supporting the production of reduced glutathione and therefore limits oxidative stress in M1 macrophages [38, 39]. Increased levels of pentose phosphate pathway metabolic intermediates satisfy the substrates need in HIF1α-prompted macrophage growth and proliferation. These data are consistent with previous studies [23, 24, 37] and lend further support to the notion that glycolysis metabolism is essential to the activation of inflammatory macrophages.

LPS-treated BMDMs were reported to tend to engage an HIF1α-dependent transcriptional program that is responsible for heightened glycolysis [40]. Metabolic mechanisms in HIF1α-deficient mice were reported to be accompanied with abolished glycolysis, decreased hepatic glucose output, and elevated gluconeogenesis [41]. On the contrast, in our study, HIF1α overexpression in the macrophages was accompanied with high mRNA levels of *Pdk1*, *Pgk1*, *Glut1*, *Gck*, and *Pkm2* (Figure 4), which was responsible for activated glycolysis. Heightened glycolysis may guarantee a competitive bioenergetic state and intensive energy for M1 macrophage polarization and also provide precursors for the production and secretion of proinflammatory cytokines [39, 42]. This process indicates the role of HIF1α in potential coordination between metabolic regulation and macrophage physiology.

5. Conclusions

In summary, we demonstrated that HIF1α activation elevates glycolysis metabolism and further induces M1 polarization of macrophages.

Conflicts of Interest

The authors declare there are no conflicts of interest.

Authors' Contributions

Ting Wang and Huiying Liu contributed equally to this work.

Acknowledgments

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References

[1] C. Cursiefen, L. Chen, L. P. Borges et al., "VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment," *The Journal of Clinical Investigation*, vol. 113, no. 7, pp. 1040–1050, 2004.

[2] A. Mantovani, S. Sozzani, M. Locati, P. Allavena, and A. Sica, "Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes," *Trends in Immunology*, vol. 23, no. 11, pp. 549–555, 2002.

[3] K. J. Moore, F. J. Sheedy, and E. A. Fisher, "Macrophages in atherosclerosis: a dynamic balance," *Nature Reviews Immunology*, vol. 13, no. 10, pp. 709–721, 2013.

[4] R. W. Kinne, R. Brauer, B. Stuhlmuller, E. Palombo-Kinne, and G. R. Burmester, "Macrophages in rheumatoid arthritis," *Arthritis Research*, vol. 2, no. 3, pp. 189–202, 2000.

[5] E. Careau and E. Y. Bissonnette, "Adoptive transfer of alveolar macrophages abrogates bronchial hyperresponsiveness," *American Journal of Respiratory Cell and Molecular Biology*, vol. 31, no. 1, pp. 22–27, 2004.

[6] E. M. Palsson-McDermott, A. M. Curtis, G. Goel et al., "Pyruvate kinase M2 regulates Hif-1α activity and IL-1β induction and is a critical determinant of the Warburg effect in LPS-activated macrophages," *Cell Metabolism*, vol. 21, no. 1, pp. 65–80, 2015.

[7] A. Sica and V. Bronte, "Altered macrophage differentiation and immune dysfunction in tumor development," *The Journal of Clinical Investigation*, vol. 117, no. 5, pp. 1155–1166, 2007.

[8] A. L. Doedens, C. Stockmann, M. P. Rubinstein et al., "Macrophage expression of hypoxia-inducible factor-1α

suppresses T-cell function and promotes tumor progression," *Cancer Research*, vol. 70, no. 19, pp. 7465–7475, 2010.

[9] A. L. Pauleau, R. Rutschman, R. Lang, A. Pernis, S. S. Watowich, and P. J. Murray, "Enhancer-mediated control of macrophage-specific arginase I expression," *Journal of Immunology*, vol. 172, no. 12, pp. 7565–7573, 2004.

[10] Z. Tan, N. Xie, H. Cui et al., "Pyruvate dehydrogenase kinase 1 participates in macrophage polarization via regulating glucose metabolism," *Journal of Immunology*, vol. 194, no. 12, pp. 6082–6089, 2015.

[11] R. K. Bruick and S. L. McKnight, "A conserved family of prolyl-4-hydroxylases that modify HIF," *Science*, vol. 294, no. 5545, pp. 1337–1340, 2001.

[12] C. Murdoch, A. Giannoudis, and C. E. Lewis, "Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues," *Blood*, vol. 104, no. 8, pp. 2224–2234, 2004.

[13] G. M. Tannahill, A. M. Curtis, J. Adamik et al., "Succinate is an inflammatory signal that induces IL-1β through HIF-1α," *Nature*, vol. 496, no. 7444, pp. 238–242, 2013.

[14] P. M. Gubser, G. R. Bantug, L. Razik et al., "Rapid effector function of memory $CD8^+$ T cells requires an immediate-early glycolytic switch," *Nature Immunology*, vol. 14, no. 10, pp. 1064–1072, 2013.

[15] G. L. Wang, B. H. Jiang, E. A. Rue, and G. L. Semenza, "Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 12, pp. 5510–5514, 1995.

[16] T. Cramer, Y. Yamanishi, B. E. Clausen et al., "HIF-1α is essential for myeloid cell-mediated inflammation," *Cell*, vol. 112, no. 5, pp. 645–657, 2003.

[17] W. G. Kaelin Jr. and P. J. Ratcliffe, "Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway," *Molecular Cell*, vol. 30, no. 4, pp. 393–402, 2008.

[18] Q. He, Z. Gao, J. Yin, J. Zhang, Z. Yun, and J. Ye, "Regulation of HIF-1α activity in adipose tissue by obesity-associated factors: adipogenesis, insulin, and hypoxia," *American Journal of Physiology. Endocrinology and Metabolism*, vol. 300, no. 5, pp. E877–E885, 2011.

[19] A. Weidemann and R. S. Johnson, "Biology of HIF-1α," *Cell Death and Differentiation*, vol. 15, no. 4, pp. 621–627, 2008.

[20] V. Nizet and R. S. Johnson, "Interdependence of hypoxic and innate immune responses," *Nature Reviews Immunology*, vol. 9, no. 9, pp. 609–617, 2009.

[21] J. S. Lewis, J. A. Lee, J. C. Underwood, A. L. Harris, and C. E. Lewis, "Macrophage responses to hypoxia: relevance to disease mechanisms," *Journal of Leukocyte Biology*, vol. 66, no. 6, pp. 889–900, 1999.

[22] K. Y. Lee, S. Gesta, J. Boucher, X. L. Wang, and C. R. Kahn, "The differential role of *Hif1β*/*Arnt* and the hypoxic response in adipose function, fibrosis, and inflammation," *Cell Metabolism*, vol. 14, no. 4, pp. 491–503, 2011.

[23] B. Kelly and L. A. O'Neill, "Metabolic reprogramming in macrophages and dendritic cells in innate immunity," *Cell Research*, vol. 25, no. 7, pp. 771–784, 2015.

[24] L. A. O'Neill and E. J. Pearce, "Immunometabolism governs dendritic cell and macrophage function," *The Journal of Experimental Medicine*, vol. 213, no. 1, pp. 15–23, 2016.

[25] X. Xue, S. Ramakrishnan, E. Anderson et al., "Endothelial PAS domain protein 1 activates the inflammatory response in the intestinal epithelium to promote colitis in mice," *Gastroenterology*, vol. 145, no. 4, pp. 831–841, 2013.

[26] S. Seki, Y. Habu, T. Kawamura et al., "The liver as a crucial organ in the first line of host defense: the roles of Kupffer cells, natural killer (NK) cells and NK1.1 Ag+ T cells in T helper 1 immune responses," *Immunological Reviews*, vol. 174, pp. 35–46, 2000.

[27] F. Tacke and H. W. Zimmermann, "Macrophage heterogeneity in liver injury and fibrosis," *Journal of Hepatology*, vol. 60, no. 5, pp. 1090–1096, 2014.

[28] C. A. Toth and P. Thomas, "Liver endocytosis and Kupffer cells," *Hepatology*, vol. 16, no. 1, pp. 255–266, 1992.

[29] S. Gordon and F. O. Martinez, "Alternative activation of macrophages: mechanism and functions," *Immunity*, vol. 32, no. 5, pp. 593–604, 2010.

[30] W. Zhang, J. M. Petrovic, D. Callaghan et al., "Evidence that hypoxia-inducible factor-1 (HIF-1) mediates transcriptional activation of interleukin-1β (IL-1β) in astrocyte cultures," *Journal of Neuroimmunology*, vol. 174, no. 1-2, pp. 63–73, 2006.

[31] W. Ertel, M. H. Morrison, A. Ayala, and I. H. Chaudry, "Hypoxemia in the absence of blood loss or significant hypotension causes inflammatory cytokine release," *The American Journal of Physiology*, vol. 269, no. 1, Part 2, pp. R160–R166, 1995.

[32] N. Takeda, E. L. O'Dea, A. Doedens et al., "Differential activation and antagonistic function of HIF-α isoforms in macrophages are essential for NO homeostasis," *Genes & Development*, vol. 24, no. 5, pp. 491–501, 2010.

[33] K. Nishi, T. Oda, S. Takabuchi et al., "LPS induces hypoxia-inducible factor 1 activation in macrophage-differentiated cells in a reactive oxygen species-dependent manner," *Antioxidants & Redox Signaling*, vol. 10, no. 5, pp. 983–995, 2008.

[34] A. Tawakol, P. Singh, M. Mojena et al., "HIF-1α and PFKFB3 mediate a tight relationship between proinflammatory activation and anerobic metabolism in atherosclerotic macrophages," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 35, no. 6, pp. 1463–1471, 2015.

[35] J. M. Olefsky and C. K. Glass, "Macrophages, inflammation, and insulin resistance," *Annual Review of Physiology*, vol. 72, pp. 219–246, 2010.

[36] H. J. Wang, Y. J. Hsieh, W. C. Cheng et al., "JMJD5 regulates PKM2 nuclear translocation and reprograms HIF-1α-mediated glucose metabolism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 1, p. 284, 2014.

[37] A. Chawla, K. D. Nguyen, and Y. P. Goh, "Macrophage-mediated inflammation in metabolic disease," *Nature Reviews Immunology*, vol. 11, no. 11, pp. 738–749, 2011.

[38] A. Haschemi, P. Kosma, L. Gille et al., "The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism," *Cell Metabolism*, vol. 15, no. 6, pp. 813–826, 2012.

[39] L. Liu, Y. Lu, J. Martinez et al., "Proinflammatory signal suppresses proliferation and shifts macrophage metabolism from Myc-dependent to HIF1α-dependent," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 6, pp. 1564–1569, 2016.

[40] M. K. Shin, L. F. Drager, Q. Yao et al., "Metabolic consequences of high-fat diet are attenuated by suppression of HIF-1α," *PLoS One*, vol. 7, no. 10, article e46562, 2012.

[41] S. Galvan-Pena and L. A. O'Neill, "Metabolic reprograming in macrophage polarization," *Frontiers in Immunology*, vol. 5, p. 420, 2014.

[42] A. J. Freemerman, A. R. Johnson, G. N. Sacks et al., "Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype," *The Journal of Biological Chemistry*, vol. 289, no. 11, pp. 7884–7896, 2014.

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Research Article

Changes in Etiologies of Hospitalized Patients with Liver Cirrhosis in Beijing 302 Hospital from 2002 to 2013

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Background. Over the last 20 years, the prevalence of hepatitis B virus (HBV) infection in China has decreased gradually due to the application of a national HBV vaccination program. In contrast, the prevalence of alcoholic liver disease (ALD), nonalcoholic fatty liver disease, autoimmune liver disease, and drug-induced liver injury has markedly increased. *Methods*. We conducted a retrospective review of 82,562 hospitalized patients diagnosed with liver cirrhosis in Beijing 302 Hospital from 2002 to 2013. *Results*. The top four etiologies of cirrhosis were HBV, HCV, ALD, and autoimmune liver disease. The percentage of HBV cirrhosis decreased from 81.53% in 2002 to 66.0% in 2013, whereas the frequency of alcoholic cirrhosis increased from 3.34% in 2002 to 8.40% in 2013. Females (84.34%) accounted for the majority of cirrhotic patients with autoimmune liver diseases. Males accounted for 80.16% of HBV cirrhosis patients and 98.02% of alcoholic cirrhosis patients. *Conclusion*. In Beijing 302 Hospital, the top four etiologies of cirrhosis were HBV, HCV, ALD, and autoimmune liver disease. Over the last 12 years, the prevalence of HBV cirrhosis has decreased gradually, whereas that of alcoholic cirrhosis has increased significantly.

1. Introduction

Recent years, viral hepatitis, especially chronic hepatitis B (CHB), is still a main reason of liver-related morbidity and mortality in China. While the prevalence of hepatitis B virus (HBV) infections fell from 10% to 7% between 1992 and 2006 as a result of a national HBV vaccination program [1]. However, due to increased national production and consumption of alcoholic beverages in China, alcoholic liver disease (ALD) is emerging as a leading cause of chronic liver disease. According to published studies, the point prevalence of ALD ranges from 2.3% to 6.1%, with a median prevalence of 4.5% in the Chinese population [2, 3]. In addition to ALD, the prevalence of autoimmune liver disease and drug-induced liver injury is increasing in China.

The aim of the present study was to investigate the variation in the disease spectrum of hospitalized patients diagnosed with liver cirrhosis in Beijing 302 Hospital between 2002 and 2013.

2. Study Population and Methods

2.1. Patients. This was a retrospective analysis of patients admitted to Beijing 302 Hospital, which is a large tertiary hospital specialized in liver diseases in Beijing. From 2002 to 2013, 82,562 patients diagnosed with liver cirrhosis were admitted to Beijing 302 Hospital. The diagnosis of cirrhosis and associated etiologies was based on clinical practice guidelines [4–9]. Patient data were obtained from the hospital's medical records. After active treatment, amelioration of symptoms, physical findings, and complications, in addition to improvements in abnormal liver function or coagulation function, was considered an improvement.

Table 1: The etiologies of 82,562 hospitalized cirrhotic patients from 2002 to 2013.

Etiology of cirrhosis	Cases	Constituent ratio (%)	Rank
Hepatitis B	58,742	71.15	1
Hepatitis C	9627	11.66	2
Alcoholic liver disease	5517	6.68	3
Autoimmune liver disease	4080	4.94	4
Cryptogenic cirrhosis	2681	3.25	5
Hepatitis B overlapping C	1119	1.36	6
Drug-induced liver injury	548	0.66	7
Hepatolenticular degeneration	128	0.16	8
Vascular obstruction disease	33	0.04	9
Nonalcoholic fatty liver disease	32	0.04	10
Bilharziasis	28	0.03	11
Cardiac cirrhosis	24	0.03	12
Malnutritional cirrhosis	3	0.00	13

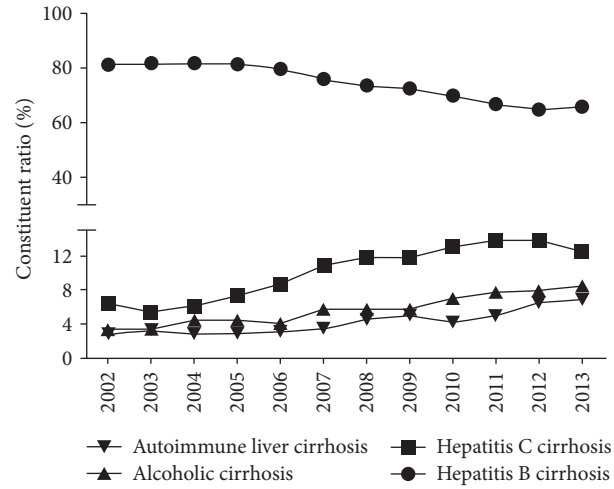

Figure 1: Changes in etiologies of cirrhosis from 2002 to 2013. For the hospitalized patients in Beijing 302 Hospital, the percentage of HBV cirrhosis decreased from 81.53% in 2002 to 66.00% in 2013. Cirrhosis due to HCV, ALD, and autoimmune liver disease increased gradually over time. Alcoholic cirrhosis increased 2.5 times from 3.34% in 2002 to 8.40% in 2013.

2.2. Statistical Analysis. Continuous variables with a normal distribution were expressed as the mean ± standard deviation (mean ± SD). Data that were not normally distributed were expressed as the median (interquartile range). An analysis of variance and SNK test were used to compare nonparametric and parametric continuous variables. Categorical variables were expressed as frequencies, with percentages. The categorical variables were analyzed by an $R \times C$ chi-square test or the Kruskal–Wallis test. Data were analyzed using SPSS version 18.0 for Windows (SPSS, Chicago, IL). Tests were two-sided, and a probability (P) value of less than 0.05 was considered statistically significant.

2.3. Ethical Approval. The study was approved by the ethics committee of Beijing 302 Hospital, and the study conformed to the Helsinki Declaration of 1977. Written informed consent was obtained from all the patients and volunteers.

3. Results

3.1. Etiologies. The etiologies of the 82,562 hospitalized cirrhotic patients are shown in Table 1. The top four etiologies of cirrhosis were HBV, HCV, ALD, and autoimmune liver diseases. Among 4080 patients with autoimmune liver cirrhosis, there were 2225 cases of autoimmune hepatitis (AIH) and 1855 cases of primary biliary cirrhosis (PBC).

3.2. Changes in Etiologies of Cirrhosis in the Last 12 Years. The most common etiologies of cirrhosis were HBV, HCV, ALD, and autoimmune liver disease, with a total of 77,966 patients diagnosed with these diseases in the past 12 years. Cirrhosis caused by them accounted for 94.43% of cases, as discussed in this paper. Supplemental Table 1 in Supplementary Material available online at https://doi.org/10.1155/2017/5605981 and Figure 1 show changes in the disease spectrum of cirrhosis patients in the past 12 years. The percentage of HBV cirrhosis decreased from 81.53% in 2002 to 66.00% in 2013. Cirrhosis due to HCV, ALD, and autoimmune liver disease increased gradually over time. Alcoholic cirrhosis increased 2.5 times from 3.34% in 2002 to 8.40% in 2013.

3.3. Gender Distribution of the Cirrhosis Patients. Figure 2 shows the gender distribution of the cirrhosis patients. Females accounted for the majority of patients with autoimmune liver cirrhosis. The percentage of female and male patients with HCV was similar. However, males accounted for the majority of HBV and alcoholic cirrhosis patients. The gender distribution of the cirrhosis groups was significantly different ($P < 0.01$).

3.4. Age of the Cirrhosis Groups. The ages of the different cirrhosis groups are indicated in Table 2. Most patients with alcoholic cirrhosis and HBV were younger than 50 years, whereas most patients with HCV cirrhosis and autoimmune liver cirrhosis were older than 50 years. There was a marked difference among groups with the P value less than 0.01.

3.5. Geographic Origin of the Patients in the Different Cirrhosis Groups. The geographic origins were divided into North China, East China, Central China, South China, Northeast China, Northwest China, and Southwest China. Figure 3 and Supplemental Table 2 show the distribution and origins of the cirrhosis groups. Most cirrhotic patients came from North China. Most patients with HBV cirrhosis, HCV cirrhosis, alcoholic cirrhosis, and autoimmune liver cirrhosis were from North China and Northeast China. However, due to the selection bias in our hospital, this distribution cannot be considered to be representative of all of China.

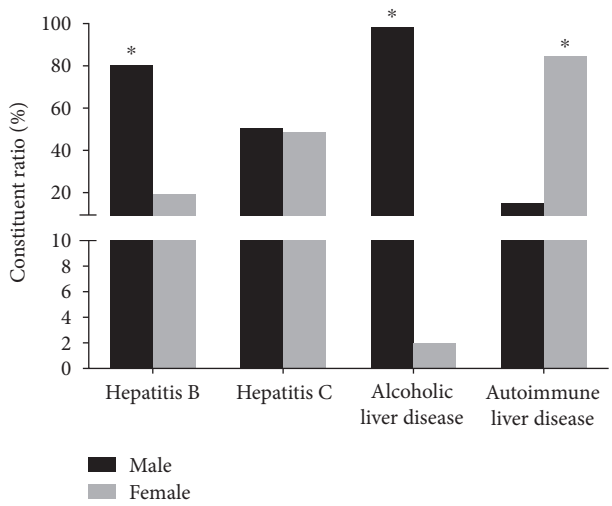

Figure 2: Gender distribution of the cirrhosis patients. For the inpatients in Beijing 302 Hospital, females accounted for the majority of patients with autoimmune liver cirrhosis. The percentage of female and male patients with HCV was comparative. Males accounted for the majority of HBV and alcoholic cirrhosis patients ($^*P < 0.01$).

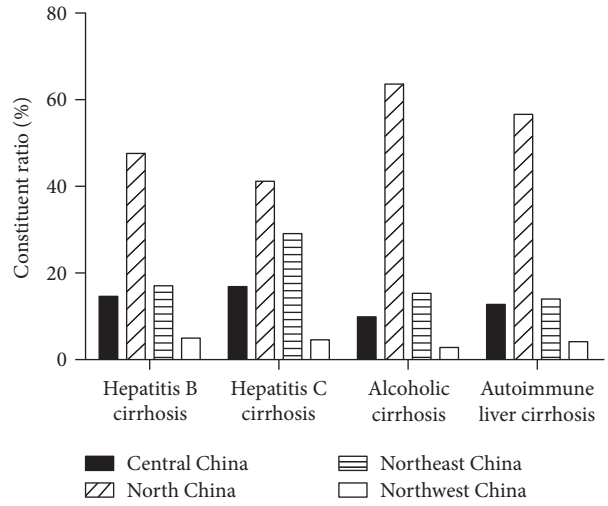

Figure 3: Geographic origin of the patients in the different cirrhosis groups. In this research, most patients with HBV cirrhosis, HCV cirrhosis, alcoholic cirrhosis, and autoimmune liver cirrhosis were from North China and Northeast China. North China includes Beijing, Tianjin, Hebei, Neimenggu, and Shanxi province. East China includes Shandong, Shanghai, Jiangsu, Anhui, Zhejiang, and Fujian province. Henan, Hubei, Hunan, and Jiangxi province are included in Central China. Guangdong, Guangxi, and Hainan are classified as South China. Northeast China consists of Heilongjiang, Jilin, and Liaoning. Northwest China comprises Ningxia, Qinghai, Shanxi, Xinjiang, and Gansu province. Southwest China includes Sichuan, Xizang, Yunnan, Guizhou, and Chongqing.

Table 2: Age of the cirrhosis groups.

	Cases	Mean	P value
Hepatitis B cirrhosis	58,742	48.19 ± 11.14	0.000
Hepatitis C cirrhosis	9627	56.73 ± 10.12	
Alcoholic cirrhosis	5517	49.62 ± 10.23	
Autoimmune liver cirrhosis	4080	56.57 ± 12.21	

Compared with hepatitis B cirrhosis or alcoholic cirrhosis, $P < 0.01$.

3.6. Prognosis of the Patients in the Different Cirrhosis Groups. Table 3 shows the prognosis of the patients in the different cirrhosis groups. After active treatment, more than 70% of HBV and HCV cirrhosis patients and nearly 80% of alcoholic and autoimmune liver cirrhosis patients showed improvements. When compared with the other cirrhosis groups, there were significant differences ($P < 0.01$).

4. Discussion

Cirrhosis refers to end-stage liver disease, which is caused by multiple factors. It is associated with various complications, including ascites, upper gastrointestinal hemorrhage, hepatic encephalopathy, and spontaneous peritonitis. It is a complex disease, with a poor prognosis. Primary hepatic cancer may occur in some cirrhosis patients. Others may require a liver transplantation because of liver cancer or decompensation of liver function.

The disease spectrum of cirrhosis in China differs from that in other countries, where cirrhosis is mainly caused by ALD and hepatitis C. For example, Moreau et al. [10] reported that alcoholic cirrhosis accounted for 66.6% of all cirrhosis cases in France, whereas hepatitis C cirrhosis, alcoholic cirrhosis overlapping with viral hepatitis, and hepatitis B cirrhosis accounted for 16%, 14.7%, and 2.7% of cases, respectively. Haukeland et al. [11] reported that among 1264 patients diagnosed with cirrhosis from January 1999 to March 2004, 53% of cases were caused by ALD and the remaining cases were due to hepatitis (12%), autoimmune liver disease (12%), hemochromatosis (4%), and nonalcoholic fatty liver disease (3%). The etiology in 16% of cases was unknown. However, based on a high incidence of diabetes mellitus, the authors concluded that cirrhosis in these cases may have been caused by nonalcoholic steatohepatitis. ALD was reported to be responsible for more than 50% of cirrhosis cases in European countries. In contrast, in our research, ALD was responsible for less than 10% of cases of cirrhosis. However, as alcohol consumption continues to increase in China, the incidence of alcoholic cirrhosis will also likely increase.

There is a high incidence of HBV infection in China. According to an epidemiological investigation of hepatitis B in 2006, the carrying rate of HBsAg was 7.18% in the population from 1 year to 59 years [12]. There are around 93 million individuals with chronic HBV infection, and about 20 million of these are chronic hepatitis patients [13]. Cirrhosis is primarily the result of HBV infection. In the present study, 71.15% of the 82,562 cirrhosis cases were caused by hepatitis B. The HBV infection rate has fallen dramatically in Chinese children since the introduction of the HBV vaccine inoculation program, and the carrying rate of HBsAg today is only 0.96% among children under 5 years [12]. The incidence of hepatitis B cirrhosis also continues to decrease annually as

Table 3: Prognosis of the patients in the different cirrhosis groups.

	Improvement	Inefficacy or death	*P* value
Hepatitis B cirrhosis	41,736 (71.05%)	17,006 (28.95%)	0.000
Hepatitis C cirrhosis	6997 (72.68%)	2630 (27.32%)	
Alcoholic cirrhosis	4365 (79.12%)	1152 (20.88%)	
Autoimmune liver cirrhosis	3261 (79.93%)	819 (20.07%)	

a result of the availability of effective antiviral drugs, such as nucleoside analogs and interferon. As shown in the present study, the percentage of hepatitis B cirrhosis declined from 81.53% in 2002 to 66.00% in 2013.

Since HBV and HCV were found, several decades have passed. This explains why most of the patients with hepatitis B and C cirrhosis in the present study were middle aged to old. The incidence of HCV can be expected to decrease further as a result of strict screening of blood products and using effective antiviral drugs.

With improvements in socioeconomic conditions, alcohol consumption has increased. According to one report in China, the production of alcohol rose from 7.113 million tons in 1984 to 30.699 million tons in 2001 [14]. There have been no nationwide epidemiological investigations of ALD. However, a regional epidemiological study found that the drinking population and incidence of ALD showed an upward trend. A survey of North China reported that the ratio of intemperants increased from 0.21% in the 1980s to 14.3% in the 1990s [15]. Since the beginning of this century, in some Chinese province, the drinking population has increased from 26.98% to 43.4% and the incidence of ALD in adults has increased from 4.3% to 6.5% [2, 16, 17]. A multiple-center study indicated that from 2000 to 2004, the hospitalized ratio of ALD to all liver diseases was 2.7%, 2.9%, 3.0%, 3.6%, and 4.4%, respectively [18]. From 2002 to 2013, there were 7422 hospitalized ALD patients in Beijing 302 Hospital, with the ratio to other liver diseases rising from 1.74% in 2002 to 4.60% in 2013 [19]. This result was similar to that of a multiple-center study in China, which will be discussed in another paper [19]. In this study, the percentage of hospitalized alcoholic cirrhosis patients increased from 3.34% in 2002 to 8.40% in 2013. Alcoholic cirrhosis had become the third most common cause of cirrhosis after hepatitis B and C cirrhosis.

The results of this paper were in accord with the real situation in China (i.e., the drinking population mainly consisted of middle-aged males). In addition, the majority of patients with autoimmune liver cirrhosis were middle-aged females. In the past, due to the absence of an effective diagnostic method, autoimmune hepatitis was difficult to diagnose. However, in recent years, it has been paid more and more attention. In the present study, the majority of cirrhosis cases were patients from North China and Northeast China. However, due to selection bias in the hospital, this distribution is not representative of China.

In this study, the top four etiologies of cirrhosis in Beijing 302 Hospital were HBV, HCV, ALD, and autoimmune liver disease. Although the prevalence of hepatitis B cirrhosis has decreased, the prevalence of alcoholic cirrhosis has increased gradually. A nationwide multiple-center study is needed to detect changes in etiologies of hospitalized patients with liver cirrhosis in the whole country.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Binxia Chang and Baosen Li contributed equally to this paper.

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References

[1] H. L. Chan and J. Jia, "Chronic hepatitis B in Asia—new insights from the past decade," *Journal of Gastroenterology and Hepatology*, vol. 26, Supplement 1, pp. 131–137, 2011.

[2] S. L. Chen, X. D. Meng, B. Y. Wang, and G. Q. Xiang, "An epidemiologic survey of ALD in some cities of Liaoning province," *Shiyong Ganzangbing Zazhi*, vol. 13, pp. 428–430, 2010.

[3] J. H. Yao, Q. D. Zhao, P. F. Xiong et al., "Investigation of ALD in ethnic groups of Yuanjiang county in Yunman," *Weichangbingxue He Ganbingxue Zazhi*, vol. 20, pp. 1137–1139, 2011.

[4] Chinese Society of Hepatoloty, Fatty Liver and Alcoholic Liver Disease Study Group, "The diagnosis and treatment guideline of alcoholic liver disease," *Linchuang Gandanbing Zazhi*, vol. 26, pp. 229–232, 2010.

[5] R. S. O'Shea, S. Dasarathy, A. J. McCullough, Practice Guideline Committee of the American Association for the Study of Liver Diseases, and Practice Parameters Committee of the American College of Gastroenterology, "Alcoholic liver disease," *Hepatology*, vol. 51, no. 1, pp. 307–328, 2010.

[6] European Association for the Study of the Liver, "EASL clinical practical guidelines: management of alcoholic liver disease," *Journal of Hepatology*, vol. 57, no. 2, pp. 399–420, 2012.

[7] Chinese Society of Hepatoloty and Chinese Society of Infecrious Diseases, Chinese Medical Association, "The prevention and treatment guidelines of chronic hepatitis B (2010)," *Shiyong Ganzangbing Zazhi*, vol. 14, pp. 81–89, 2011.

[8] Chinese Society of Hepatoloty, Chinese Society of Infecrious and Parasitic Diseases, Chinese Medical Association, "The prevention and treatment guidelines of hepatitis C," *Chuanranbing Xinxi*, vol. 17, pp. 9–14, 2004.

[9] European Association for the Study of the Liver, "EASL clinical practice guidelines: management of cholestatic liver diseases," *Journal of Hepatology*, vol. 51, pp. 237–267, 2009.

[10] R. Moreau, P. Delègue, F. Pessione et al., "Clinical characteristics and outcome of patients with cirrhosis and refractory ascites," *Liver International*, vol. 24, pp. 457–464, 2004.

[11] J. W. Haukeland, I. Lorgen, L. T. Schreiner et al., "Incidence rates and causes of cirrhosis in a Norwegian population," *Scandinavian Journal of Gastroenterology*, vol. 42, no. 12, pp. 1501–1508, 2007.

[12] X. Liang, S. Bi, W. Yang et al., "Evaluation of the impact of hepatitis B vaccination among children born during 1992–2005 in China," *The Journal of Infectious Diseases*, vol. 200, no. 1, pp. 39–47, 2009.

[13] L. FM and H. Zhuang, "Management of hepatitis B in China," *Chinese Medical Journal*, vol. 122, pp. 3-4, 2009.

[14] Z. Hui, "Epidemiology of alcoholic liver disease," *Chinese Journal of Hepatology*, vol. 11, p. 412, 2003.

[15] Y. M. Li, W. X. Chen, C. H. Yu et al., "The epidemiological investigation of alcoholic liver disease in Zhejiang province," *Chinese Journal of Hepatology*, vol. 11, pp. 647–649, 2003.

[16] X. L. Lu, M. Tao, J. Y. Luo, Y. Geng, H. L. Zhao, and P. Zhao, "The epidemiological investigation of drinking and liver disease," *Chinese Journal of Hepatology*, vol. 10, pp. 467-468, 2002.

[17] S. L. Huang, S. Q. Dai, X. H. Zhang, Y. J. Yu, M. L. Tan, and C. G. Yi, "The epidemiological investigation of alcoholic liver disease in Hunan province," *Zhongguo Yishi Zazhi*, vol. 7, pp. 426-427, 2005.

[18] National investigation group of alcoholic liver disease, "National multiple investigation and analysis of alcoholic liver disease," *Chinese Journal of Digestion*, vol. 27, pp. 31–34, 2007.

[19] B. X. Chang, B. S. Li, A. Huang et al., "Changes of four common non-infectious liver diseases for the hospitalized patients in Beijing 302 hospital from 2002 to 2013," *Alcohol*, vol. 54, pp. 61–65, 2016.

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Review Article

IL-33-ST2 Axis in Liver Disease: Progression and Challenge

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The new member of the IL-1 family, interleukin-33 (IL-33), participates in the progression of a variety of diseases through binding with its receptor ST2. Recently, much clinical evidence and experimental data have indicated that IL-33 is associated with various liver diseases. This review primarily addresses the relationship between IL-33 and several hepatic diseases. IL-33 can alleviate high-fat diet- (HFD-) induced hepatic steatosis and insulin resistance, and IL-33 acts as an alarmin, which quickly triggers the immune system to respond to virus invasion and toxic damage to the liver. However, when liver injury is chronic, IL-33 promotes Th2 reactions and hepatic stellate cell (HSC) activity, facilitating progression to liver fibrosis. The complicated functions of IL-33 should be considered before its clinical application.

1. Brief Introduction of IL-33

Interleukin-33 (IL-33) was originally discovered (as clone DVS 27) in a study of canine vasospastic cerebral arteries after subarachnoid haemorrhage and received attention due to its highly upregulated expression [1]. IL-33 was then identified as NF-HEV expressed in human high endothelial venules and shown to function as a nuclear factor [2]. In 2005, Schmitz and coworkers matched the sequence structure of IL-33 with the IL-1 cytokine family, and IL-33 was successfully classified as part of the IL-1 family and named IL-33. The IL-33 gene is located on chromosome 9p24.1 in humans and on the syntenic chromosome 19qC1 region in mice. IL-33 cDNA encodes 270 and 266 amino acid polypeptides in humans and mice, respectively, and the full-length proteins have respective masses of 30 and 29.9 kDa. Caspase-1 can cleave IL-33 to a mass of 18 kDa, and the amino acid similarity between human and mouse IL-33 is up to 55% [3]. IL-33 mRNA can be found in multiple cells and tissues in both humans and mice; at the protein level, IL-33 is broadly expressed in endothelial cells, epithelial cells, smooth muscle cells, and several organs, such as the lung and central nervous system [4–6].

The excellent work by Schmitz et al. indicated that IL-33 is the ligand of the orphan receptor ST2, which is a member of the IL-1 receptor family [3]. ST2 protein exists in at least 3 isoforms through diverse splicing: a transmembrane form (ST2L), a soluble form (sST2), and a novel variant [7]. ST2 is expressed by several types of haematopoietic cells [3]. In addition, ST2 is selectively and stably expressed on the surface of T helper 2 (T_H2) cells but not on that of T_H1 or regulatory T (Treg) cells [8, 9]. IL-33 binds ST2, which then connects with IL-1R accessory protein (IL-1RcAP) to form a heterodimeric complex [10]. sST2 is considered a decoy receptor [11] that competes with ST2L for IL-33 binding, subsequently blocking the IL-33 signalling pathway [12]. Single immunoglobulin IL-1R-related molecule (SIGIRR) associated with ST2 has the ability to inhibit IL-33/ST2-mediated signalling [13].

Binding of IL-33 with its receptor recruits myeloid differentiation primary response protein 88 (myD88), IL-1R-associated kinase 1 (IRAK1), and IRAK4 to the receptor through IL-1RAcP's Toll-interleukin 1 receptor (TIR) domain [3]. Subsequently, certain downstream signalling molecules are activated, including nuclear factor-κB (NF-κB) [14], inhibitor of NF-κB-α (IκBα), extracellular signal-regulated

kinase (ERK), p38, serine/threonine protein kinase Akt (protein kinase B), and c-Jun N-terminal kinase (JNK) [15].

Based on the conserved homeodomain-like helix-turn-helix motif in the N-terminal portion of IL-33 and on the nuclear localization signal, IL-33 may play dual roles. First, it behaves as a traditional cytokine activating downstream signalling, and second, it acts as an intracellular nuclear factor with transcriptional regulatory properties [16]. Among the numerous biological functions of IL-33, the best known is promoting polarization of naïve T cells to T_H2-type immune response cells, and it can act directly on T_H2 cells to increase IL-5 and IL-13 production [3]. Furthermore, IL-33 has been identified as a selective Th2 chemoattractant [17]. In addition to T_H2 cells, IL-33 also interacts with many other immune cells. For example, IL-33 attenuates TLR4-induced downregulation of CXCR2 and chemotaxis in neutrophils [18], and IL-33/ST2 signalling participates in alternatively activated M2 macrophage polarization in macrophages [19]. Another well-known function of IL-33 is that of an "alarmin"; under cell damage (necrosis) or mechanical injury conditions, active full-length IL-33 can be released rapidly to alter the immune system response [20].

IL-33 is associated with a variety of diseases. Whether IL-33 promotes or inhibits disease progression depends on the type of disease. In asthma patients, IL-33 expression was elevated significantly [5, 21], and in a mouse model of asthma, $ST2^{-/-}$ mice developed attenuated airway inflammation [22]. IL-33 levels were elevated in sera and synovial fluid samples of rheumatoid arthritis (RA) patients and were correlated with the activity of the disease [23]. The serum level of IL-33 decreased after anti-TNF treatment and was correlated with the production of IgM and RA-related autoantibodies [24]. IL-33 expression was significantly increased in the inflamed mucosa of inflammatory bowel disease (IBD) patients as well as in colitis mice induced by dextran sulphate sodium (DSS) [25, 26]. IL-33 expression in the brain was downregulated in Alzheimer's disease (AD) cases compared with controls [27], and a similar conclusion was drawn by another study [28]. Recently, it has been reported that IL-33 can ameliorate AD-like pathology and cognitive decline, and the authors proposed that IL-33 is a promising potential treatment for AD [29]. As the IL-33 decoy receptor, the sST2 level rose immediately after acute myocardial infarction [30], and the serum ST2 level was identified as a novel biomarker for neurohormonal activation in heart failure patients [31]. On the other hand, researchers have found that in ischemia/reperfusion model rats, IL-33 prevented cardiomyocyte apoptosis and enhanced cardiac function through ST2 signalling [32].

2. IL-33 and Liver Disease

The relationship between IL-33 and liver disease, as well as its role in the development of liver disease, has attracted the attention of an increasing number of researchers. So far, IL-33 has been found to be involved in a variety of liver diseases, including fatty liver disease, hepatitis, liver fibrosis, and cirrhosis, along with other hepatic diseases (Table 1).

2.1. IL-33 and Fatty Liver Disease. As recently as 20 years ago, researchers realized that in many industrialized countries, nearly a quarter of adults had excessive fat accumulation in the liver, and fatty liver was a vital risk factor for serious liver disease [33]. Studies regarding the role of IL-33 in fatty liver disease have primarily focused on nonalcoholic fatty liver disease (NAFLD). The spectrum of NAFLD ranges from fatty liver alone to nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis and its associated complications without a history of heavy alcohol consumption [34, 35]. NAFLD is commonly found in type 2 diabetes and obese patients, and insulin resistance is closely related to NAFLD development and prognosis [36]. One study showed that *in vitro* administration of IL-33 into adipose tissue cultures induced Th2 cytokine production (IL-5, IL-13, and IL-10) and downregulated the expression of adipogenic genes. Administration of IL-33 to genetically obese diabetic (ob/ob) mice resulted in reduced adiposity and improved glucose and insulin tolerance [37].

Because of the regulatory role played by IL-33 in lipid metabolism, IL-33 may have a close relationship with fatty liver. An NAFLD mouse model was successfully constructed by feeding mice with a high-fat diet (HFD) [38]. The results of a recent study showed that a HFD given to mice for 20 weeks induced upregulation of both IL-33 and ST2 mRNA and proteins. Furthermore, treatment with IL-33 alleviated HFD-induced hepatic steatosis, reduced serum alanine aminotransferase (ALT) levels, and ameliorated insulin resistance and glucose intolerance. Notably, the researchers found that the serum IL-33 levels and IL-33 mRNA levels in the liver were higher in NAFLD patients. The group also confirmed that IL-33 promoted a Th2 response, M2 macrophage activation, and fatty acid metabolism gene expression in the liver [39]. Meanwhile, in another study, $ST2^{-/-}$ mice fed with a HFD exhibited increased weight gain and visceral adipose tissue, but the deletion of ST2 ameliorated hepatic steatosis and inflammation [40]. Because the IL-33/ST2 axis may have a disputably beneficial effect on fatty liver, more studies are needed to clarify its mechanism and determine its therapeutic value.

2.2. IL-33 and Hepatitis. Multiple aetiological factors can lead to hepatitis, such as viral infection, alcohol abuse, toxicants, drugs, and autoimmunity. Changes in IL-33 expression in viral hepatitis and fulminant hepatitis triggered by toxins have been recently reported, suggesting that IL-33 may participate in different types of hepatitis. Approximately 70% of hepatitis C virus- (HCV-) infected patients cannot completely clear the HCV and eventually can develop persistent chronic infection. Cirrhosis and hepatocellular carcinoma can develop in many of these patients [41, 42]. What is the role of IL-33 in this disease? Wang et al. drew conclusions by comparing chronic hepatitis C (CHC) patients, spontaneously resolved HCV (SR-HCV) patients, and healthy controls (HCs). They found that serum IL-33 levels in CHC patients were significantly higher than those in HC and SR-HCV patients, while IL-33 levels decreased after treatment with interferon for 12 weeks, and this decrease was correlated with ALT and aspartate aminotransferase

Table 1: Studies on the roles of IL-33 and ST2 in liver diseases.

Disease	Result	Ref
Fatty liver disease	(i) The mRNA and protein levels of both IL-33 and ST2 were increased in the mouse model of HFD-induced hepatic steatosis, and treatment with IL-33 alleviated hepatic steatosis.	[39]
	(ii) $ST2^{-/-}$ mice fed with HFD exhibited increased weight gain, severe hepatic steatosis, and inflammation.	[40]
	(iii) The IL-33 mRNA levels in serum and liver were increased in NAFLD patients.	[39]
Hepatitis	(i) Serum IL-33 levels in CHC patients were significantly higher than those in HCs while decreased after treatment with interferon and were correlated with the ALT and AST concentrations.	[43, 44]
	(ii) Serum IL-33 concentrations in CHC patients were positively correlated with the levels of serum HCV RNA.	[45]
	(iii) CHB patients with high serum ALT concentrations showed higher serum IL-33 and ST2 levels.	[47]
	(iv) In poly(I:C)-induced murine fulminant hepatitis, the expression of IL-33 was upregulated, and in NK-depleted poly(I:C)-treated mice, liver injury was severe while NKT-deficient mice showed hepatoprotection against poly(I:C)-induced hepatitis accompanied by an increased number of IL-33-expressing hepatocytes.	[48]
	(v) IL-33-knockout mice infected by LCMV produced fewer IFN-γ^+ $\gamma\delta$ T and NK cells, and rIL-33 treatment facilitated IFN-γ-producing $\gamma\delta$ T and NK cells and inhibited IL-17^+ $\gamma\delta$ T cells.	[49, 50]
	(vi) IL-33 and ST2 levels were increased in mouse liver after Ad infection. Injection of rIL-33 resulted in a decrease in serum ALT levels and the number of Councilman bodies in the liver; meanwhile, Treg cells were upregulated and TNF-α levels in the liver decreased.	[51]
	(vii) ST2-deficient mice developed severer hepatitis induced by Con A with a higher number of mononuclear cells and higher level of proinflammatory cytokines in the liver. IL-33 also suppressed caspase-3 activation and BAX expression as well as enhanced Bcl-2 expression in the liver.	[52]
	(viii) NKT-deficient mice performed resistant to Con A-induced hepatitis and lacked IL-33 expression in liver cells.	[53]
	(ix) IL-33 expression in hepatocytes was blocked during Con A-induced acute hepatitis in TRAIL-deficient mice.	[54]
	(x) The severity of liver injury in IL-$33^{-/-}$ mice was positively correlated with the levels of TNF-α and IL-1β and the number of NK cells infiltrating into the liver.	[55]
	(xi) rIL-33 exacerbated Con A-induced hepatitis, while pretreatment of an IL-33-blocking antibody exhibited a protective effect.	[56]
Liver fibrosis/cirrhosis	(i) In mouse and human fibrotic livers, IL-33 and ST2 mRNA was overexpressed and the major sources of IL-33 were HSCs.	[60]
	(ii) IL-33 led to activation and accumulation of ILC2 through ST2 signalling in the liver, and activated ILC2 produced IL-13; then, IL-13 initiated activation and differentiation of HSCs.	[63]
	(iii) In ST2-deficient mice with liver fibrosis, the activation of HSCs was decreased and *in vitro* HSCs activated by rIL-33 release IL-6, TGF-β, α-SMA, and collagen.	[64]
	(iv) Serum IL-33 levels of PBC patients were positively correlated with the severity of PBC.	[66]
Others	(i) A high level of IL-33 mainly produced by $CD8^+$ $CD62L^-$ $KLRG1^+$ $CD107a^+$ T cells might indicate prolonged patient survival.	[68]
	(ii) A high level of serum sST2 is regarded as a negative HCC prognostic factor.	[69]
	(iii) IL-33 presented a significant protective effect on liver ischemia/reperfusion mouse model with attenuated liver damage and limited inflammatory activity.	[72, 73]
	(iv) IL-33 participated in hepatic granuloma pathology during *Schistosoma japonicum* infection.	[70]
	(v) In *Leishmania donovani*-infected liver mice, the IL-33/ST2 axis suppressed Th1 response and patients with visceral leishmaniasis showed higher serum IL-33 levels.	[71]

(AST) levels in CHC patients [43]. Meanwhile, Hamdi and coworkers obtained similar results [44]. In another study, serum HCV RNA was also detected, and it was found that serum IL-33 concentrations were positively correlated with the levels of serum HCV RNA [45].

As a pathogenic factor, IL-33 plays a role not only in CHC but also in chronic hepatitis B (CHB). Hepatitis B virus (HBV) is another major cause of chronic liver disease. After HBV infection, most adults can clear the virus spontaneously, but nearly 5% of infected adults and more than 90% of infected infants and young children will develop chronic infection [46]. CHB also has a risk of progressing to liver cirrhosis and hepatocellular carcinoma [41, 42]. One study showed that the serum IL-33 and ST2 levels were elevated as serum ALT concentrations increased in CHB patients compared to HBV carriers, HCs, and CHB patients with low ALT levels [47]. All these results suggest that IL-33 is associated with liver damage. Therefore, IL-33 has been proposed to function as an alarmin to alert the immune system of tissue damage following infection.

To facilitate research, many researchers have explored the relationship between IL-33 and hepatitis in animal hepatitis models. Arshad et al. detected IL-33 expression in murine fulminant hepatitis induced by poly(I:C), a Toll-like receptor (TLR3) viral mimetic, and by pathogenic mouse hepatitis virus (L2-MHV3). Their results showed that in both

hepatitis mouse models, the expression of IL-33 was upregulated and hepatocyte-specific IL-33 expression was downregulated in natural killer cell- (NK-) depleted poly(I:C)-treated mice with severe liver injury, while natural killer T cell- (NKT-) deficient mice exhibited hepatoprotection against poly(I:C)-induced hepatitis accompanied by an increased number of IL-33-expressing hepatocytes compared with wild-type (WT) controls [48]. Lymphocytic choriomeningitis virus- (LCMV-) infected IL-33-knockout mice were used in another study, and the study indicated that IL-33 deficiency resulted in fewer IFN-γ^+ $\gamma\delta$ T and NK cells. In contrast, recombinant IL-33 (rIL-33) facilitated IFN-γ-producing $\gamma\delta$ T and NK cells and inhibited IL-17$^+$ $\gamma\delta$ T cells, revealing a role of IL-33 in regulating innate IFN-γ production and antiviral responses in LCMV-infected hepatitis [49, 50]. Liang et al. used another virus, adenovirus (Ad), to induce hepatitis. During the first week of Ad infection, a continuous increase in IL-33 and ST2 expression was observed in mouse livers. Injection of rIL-33 resulted in a decrease in serum ALT levels and the number of Councilman bodies in the liver. These changes were correlated with the upregulation of Treg cells and downregulation of macrophages, dendritic cells, and NK cells in the liver, and at the same time, TNF-α expression was inhibited by IL-33 in hepatic T cells and macrophages, and TNF-α levels in the liver decreased [51].

Another focus of the relationship between IL-33 and hepatitis is the protective role of the IL-33/ST2 axis in concanavalin A- (Con A-) induced hepatitis. A study researched by Volarevic and coworkers indicated that severe hepatitis developed in Con A-treated ST2-deficient mice, and these mice exhibited a high number of mononuclear cells in the liver and a high level of proinflammatory cytokines (TNF-α and IFN-γ). In contrast, in WT mice, the number of CD4$^+$Foxp3$^+$ cells was statistically higher. Furthermore, injection of IL-33 into WT mice attenuated liver damage and increased the number of liver CD4$^+$Foxp3$^+$ cells. IL-33 also suppressed caspase-3 activation and the expression of BAX and enhanced Bcl-2 expression in the liver [52]. Interestingly, NKT-deficient mice were also resistant to Con A-induced hepatitis and no longer expressed IL-33 in liver cells following Con A administration, while IL-33 was overexpressed in normal mice [53]. Meanwhile, IL-33 expression in hepatocytes was also blocked during Con A-induced acute hepatitis in tumour necrosis factor-related apoptosis-inducing ligand- (TRAIL-) deficient mice, and IL-33-deficient mice exhibited more severe Con A-induced liver injury than WT mice [54]. Furthermore, the severity of liver injury in IL-33$^{-/-}$ mice was positively correlated with TNF-α and IL-1β levels and the number of NK cells infiltrating into the liver [55]. The majority of studies in this area support the view that IL-33 protects against Con A-induced hepatitis, and this protection involves a variety of immune cells (Treg, NK, and NKT cells) and molecules (IFN-γ and TRAIL). However, the opposite result was obtained in one study: treatment of rIL-33 exacerbated Con A-induced hepatitis, but pretreatment with an IL-33-blocking antibody exhibited a protective effect, likely by suppressing the late stage of T cell and NKT cell activation and decreasing IFN-γ production [56]. More studies are needed to determine whether IL-33 protects or aggravates hepatitis induced by drugs and to elucidate the reasons for this discrepancy.

2.3. IL-33 and Liver Fibrosis (and Cirrhosis). Liver fibrosis and its end-stage form, cirrhosis, are the common final pathway for virtually all chronic liver diseases. Accumulation of extracellular matrix (ECM) rich in fibrillar collagens (mainly collagen I and collagen III) is the characteristic of advanced fibrosis, and it is associated with liver failure, portal hypertension, and a high risk of liver cancer [57, 58]. In the course of chronic hepatitis and progression to cirrhosis, in addition to persistent inflammatory infiltrate, a Th2-polarized immune response always occurs. Th1 cytokines lead to a rapid and intense inflammatory response while causing little fibrosis. In contrast, Th2 cytokines, such as IL-13, promote hepatic stellate cell (HSC) proliferation, transforming growth factor-β (TGF-β) synthesis, and fibrogenesis [59].

Based on the crucial role of Th2 cytokines in liver fibrosis formation and the pro-Th2 activity of IL-33, the relationship between IL-33 and liver fibrosis has received much attention. One study has shown that in mouse and human fibrotic livers, IL-33 and ST2 mRNA is overexpressed. Moreover, IL-33 expression was correlated with collagen expression, and the major source of IL-33 in fibrotic livers was HSCs [60]. Another study deeply explored the mechanism of IL-33 in promoting the pathogenesis of hepatic fibrosis; this mechanism involved a new type of lymphocyte, innate lymphoid cell type 2 (ILC2), which expresses IL33R-ST2. IL-33-responsive ILC2 cells are widely distributed in the mesenteric lymph nodes, spleen, and liver of mice and produce several Th2 cytokines, such as IL-4, IL-5, and IL-13 [61, 62]. The study also revealed that in hepatic fibrosis, IL-33 expression was elevated, and excess ECM deposition was sufficiently driven by IL-33 alone in the liver. Furthermore, IL-33$^{-/-}$ mice displayed a significant amelioration of experimental fibrosis. IL-33 led to activation and accumulation of ILC2 cells through ST2 signalling in the liver. Activated ILC2 cells produced IL-13, and then, IL-13 initiated activation and differentiation of HSCs via the IL-4Rα-STAT6 transcription factor-dependent pathway [63]. Meanwhile, another study showed that activation of HSCs was decreased in ST2-deficient liver fibrosis mice and that HSCs were activated by rIL-33 *in vitro*, releasing IL-6, TGF-β, α-SMA, and collagen [64].

Although earlier we introduced the idea that IL-33 could alleviate HFD-induced hepatic steatosis and insulin resistance, it has been verified that in diet-induced NASH, IL-33-mediated aggravation of hepatic fibrosis was dependent on the ST2 signalling pathway [39]. In primary biliary cirrhosis (PBC), an autoimmune liver disease with complications such as cirrhosis, liver failure, and hepatoma carcinoma [65], the serum IL-33 level of patients was positively correlated with severity [66]. In general, IL-33 showed a potential promotive effect on liver fibrosis.

There are also research teams studying the role of IL-33 in other liver diseases; for example, oncogenesis and progression of hepatocellular carcinoma (HCC) are associated with aberrant IL-33 expression [67], and upregulation of IL-33,

primarily produced by $CD8^+$ $CD62L^-$ $KLRG1^+$ $CD107a^+$ T cells, may indicate prolonged patient survival [68]. However, high levels of serum sST2 were considered a negative HCC prognostic factor [69]. During *Schistosoma japonicum* infection, IL-33 participated in hepatic granuloma pathology [70]; in the liver of *Leishmania donovani*-infected mice, the IL-33/ST2 axis suppressed Th1 response, and patients with visceral leishmaniasis exhibited higher serum IL-33 levels [71]. IL-33 exhibited a significant protective effect on a liver ischemia/reperfusion mouse model and attenuated liver damage and limited inflammatory activity [72, 73].

3. Conclusion and Expectations

It has been nearly 30 years since the discovery of IL-33, and many studies have been performed to determine the molecular structure, distribution, receptor, and signalling pathway of IL-33. Knowledge regarding the molecular basis of IL-33 signalling is relatively comprehensive. Nevertheless, the role of IL-33 is different in various liver diseases. IL-33 can attenuate hepatic steatosis and act as an alarmin by quickly triggering the immune system to respond to virus invasion and toxicant-induced damage, thus leading to a protective effect on viral hepatitis and Con A-induced liver injury. However, IL-33 promotes Th2 reactions and HSC activity, facilitating the progression to liver fibrosis. Therefore, evidence suggests that when acute and massive liver damage occurs, the release of IL-33 by injured hepatocytes might be a protective mechanism, while in chronic injury, IL-33 plays the role of a hepatic fibrosis-enhancing factor. Thus, it is necessary to judge and weigh the opposing functions of IL-33 before clinical application [74].

Although great progress has been made in understanding the relationship between IL-33 and liver disease, the majority of studies are still based on correlations between IL-33 expression and liver disease. Studies on the specific mechanism are not thorough or sufficiently comprehensive, and several experimental results suggest opposite conclusions. Hence, more studies are required to fully understand the role of IL-33 in the regulation of liver disease and its signalling pathways and regulatory networks.

Moreover, we also hope to expand the study of IL-33 to more liver diseases and find more potential therapeutic applications of IL-33. For instance, alcoholic liver disease (ALD) exhibits a disease progression similar to that of NAFLD (from simple fatty liver to alcoholic hepatitis, cirrhosis, and even HCC), and its incidence is rapidly increasing. ALD is becoming an important cause of chronic liver disease worldwide, while (except for abstinence) ALD lacks therapeutic drugs with definite efficacy [75]. Therefore, it is very likely that the role of IL-33 in ALD will be discovered and provide a new treatment approach for ALD. Further study of IL-33, a novel cytokine, could establish a new field of research on the mechanisms and treatment of liver disease.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding this article.

Authors' Contributions

Zijian Sun and Binxia Chang participated in the design and writing of the paper; they contributed equally to the work. Jiyuan Zhang and Zhengsheng Zou participated in the design and literature review. Miaomiao Gao provided substantial advice in designing the paper.

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References

[1] H. Onda, H. Kasuya, K. T. Hori et al., "Identification of genes differentially expressed in canine vasospastic cerebral arteries after subarachnoid hemorrhage," *Journal of Cerebral Blood Flow & Metabolism*, vol. 19, no. 11, pp. 1279–1288, 1999.

[2] E. S. Baekkevold, M. Roussigné, T. Yamanaka et al., "Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules," *American Journal of Pathology*, vol. 163, no. 1, pp. 69–79, 2003.

[3] J. Schmitz, A. Owyang, E. Oldham et al., "IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines," *Immunity*, vol. 23, no. 5, pp. 479–490, 2005.

[4] C. Moussion, N. Ortega, and J. P. Girard, "The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells *in vivo*: a novel 'alarmin'?," *PLoS One*, vol. 3, no. 10, article e3331, 2008.

[5] D. Prefontaine, S. Lajoie-Kadoch, S. Foley et al., "Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells," *Journal of Immunology*, vol. 183, no. 8, pp. 5094–5103, 2009.

[6] C. A. Hudson, G. P. Christophi, R. C. Gruber, J. R. Wilmore, D. A. Lawrence, and P. T. Massa, "Induction of IL-33 expression and activity in central nervous system glia," *Journal of Leukocyte Biology*, vol. 84, no. 3, pp. 631–643, 2008.

[7] K. Tago, T. Noda, M. Hayakawa et al., "Tissue distribution and subcellular localization of a variant form of the human ST2 gene product, ST2V," *Biochemical and Biophysical Research Communications*, vol. 285, no. 5, pp. 1377–1383, 2001.

[8] D. Xu, W. L. Chan, B. P. Leung et al., "Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells," *The Journal of Experimental Medicine*, vol. 187, no. 5, pp. 787–794, 1998.

[9] S. Lecart, N. Lecointe, A. Subramaniam et al., "Activated, but not resting human Th2 cells, in contrast to Th1 and T regulatory cells, produce soluble ST2 and express low levels of ST2L at the cell surface," *European Journal of Immunology*, vol. 32, no. 10, pp. 2979–2987, 2002.

[10] A. A. Chackerian, E. R. Oldham, E. E. Murphy, J. Schmitz, S. Pflanz, and R. A. Kastelein, "IL-1 receptor accessory protein and ST2 comprise the IL-33 receptor complex," *Journal of Immunology*, vol. 179, no. 4, pp. 2551–2555, 2007.

[11] G. Bandara, M. A. Beaven, A. Olivera, A. M. Gilfillan, and D. D. Metcalfe, "Activated mast cells produce soluble ST2, a decoy receptor for IL-33," *Journal of Allergy and Clinical Immunology*, vol. 135, no. 2, article AB64, 2015.

[12] H. Hayakawa, M. Hayakawa, A. Kume, and S. Tominaga, "Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation," *The Journal of Biological Chemistry*, vol. 282, no. 36, pp. 26369–26380, 2007.

[13] K. Bulek, S. Swaidani, J. Qin et al., "The essential role of single Ig IL-1 receptor-related molecule/toll IL-1R8 in regulation of Th2 immune response," *Journal of Immunology*, vol. 182, no. 5, pp. 2601–2609, 2009.

[14] C. Bouffi, M. Rochman, C. B. Zust et al., "IL-33 markedly activates murine eosinophils by an NF-κB-dependent mechanism differentially dependent upon an IL-4-driven autoinflammatory loop," *Journal of Immunology*, vol. 191, no. 8, pp. 4317–4325, 2013.

[15] C. K. Wong, K. M. Leung, H. N. Qiu, J. Y. Chow, A. O. Choi, and C. W. Lam, "Activation of eosinophils interacting with dermal fibroblasts by pruritogenic cytokine IL-31 and alarmin IL-33: implications in atopic dermatitis," *PLoS One*, vol. 7, no. 1, article e29815, 2012.

[16] V. Carriere, L. Roussel, N. Ortega et al., "IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor *in vivo*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 1, pp. 282–287, 2007.

[17] M. Komai-Koma, D. Xu, Y. Li, A. N. McKenzie, I. B. McInnes, and F. Y. Liew, "IL-33 is a chemoattractant for human Th2 cells," *European Journal of Immunology*, vol. 37, no. 10, pp. 2779–2786, 2007.

[18] J. C. Alves-Filho, F. Sonego, F. O. Souto et al., "Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection," *Nature Medicine*, vol. 16, no. 6, pp. 708–712, 2010.

[19] M. Kurowska-Stolarska, B. Stolarski, P. Kewin et al., "IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation," *Journal of Immunology*, vol. 183, no. 10, pp. 6469–6477, 2009.

[20] C. Cayrol and J. P. Girard, "IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy," *Current Opinion in Immunology*, vol. 31, pp. 31–37, 2014.

[21] D. Prefontaine, J. Nadigel, F. Chouiali et al., "Increased IL-33 expression by epithelial cells in bronchial asthma," *The Journal of Allergy and Clinical Immunology*, vol. 125, no. 3, pp. 752–754, 2010.

[22] M. Kurowska-Stolarska, P. Kewin, G. Murphy et al., "IL-33 induces antigen-specific IL-5$^+$ T cells and promotes allergic-induced airway inflammation independent of IL-4," *Journal of Immunology*, vol. 181, no. 7, pp. 4780–4790, 2008.

[23] Y. Matsuyama, H. Okazaki, H. Tamemoto et al., "Increased levels of interleukin 33 in sera and synovial fluid from patients with active rheumatoid arthritis," *The Journal of Rheumatology*, vol. 37, no. 1, pp. 18–25, 2010.

[24] R. Mu, H. Q. Huang, Y. H. Li, C. Li, H. Ye, and Z. G. Li, "Elevated serum interleukin 33 is associated with autoantibody production in patients with rheumatoid arthritis," *The Journal of Rheumatology*, vol. 37, no. 10, pp. 2006–2013, 2010.

[25] J. B. Seidelin, J. T. Bjerrum, M. Coskun, B. Widjaya, B. Vainer, and O. H. Nielsen, "IL-33 is upregulated in colonocytes of ulcerative colitis," *Immunology Letters*, vol. 128, no. 1, pp. 80–85, 2010.

[26] M. Sun, C. He, W. Wu et al., "Hypoxia inducible factor-1α-induced interleukin-33 expression in intestinal epithelia contributes to mucosal homeostasis in inflammatory bowel disease," *Clinical and Experimental Immunology*, vol. 187, no. 3, pp. 428–440, 2017.

[27] J. Chapuis, D. Hot, F. Hansmannel et al., "Transcriptomic and genetic studies identify IL-33 as a candidate gene for Alzheimer's disease," *Molecular Psychiatry*, vol. 14, no. 11, pp. 1004–1016, 2009.

[28] J. T. Yu, J. H. Song, N. D. Wang et al., "Implication of IL-33 gene polymorphism in Chinese patients with Alzheimer's disease," *Neurobiology of Aging*, vol. 33, no. 5, pp. 1014.e11–1014.e14, 2012.

[29] A. K. Fu, K. W. Hung, M. Y. Yuen et al., "IL-33 ameliorates Alzheimer's disease-like pathology and cognitive decline," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 19, pp. E2705–E2713, 2016.

[30] M. Shimpo, D. A. Morrow, E. O. Weinberg et al., "Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction," *Circulation*, vol. 109, no. 18, pp. 2186–2190, 2004.

[31] E. O. Weinberg, M. Shimpo, S. Hurwitz, S. Tominaga, J. L. Rouleau, and R. T. Lee, "Identification of serum soluble ST2 receptor as a novel heart failure biomarker," *Circulation*, vol. 107, no. 5, pp. 721–726, 2003.

[32] K. Seki, S. Sanada, A. Y. Kudinova et al., "Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling," *Circulation. Heart Failure*, vol. 2, no. 6, pp. 684–691, 2009.

[33] H. Z. Lin, S. Q. Yang, C. Chuckaree, F. Kuhajda, G. Ronnet, and A. M. Diehl, "Metformin reverses fatty liver disease in obese, leptin-deficient mice," *Nature Medicine*, vol. 6, no. 9, pp. 998–1003, 2000.

[34] C. A. Matteoni, Z. M. Younossi, T. Gramlich, N. Boparai, Y. C. Liu, and A. J. McCullough, "Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity," *Gastroenterology*, vol. 116, no. 6, pp. 1413–1419, 1999.

[35] S. Alam, G. Mustafa, M. Alam, and N. Ahmad, "Insulin resistance in development and progression of nonalcoholic fatty liver disease," *World Journal of Gastrointestinal Pathophysiology*, vol. 7, no. 2, pp. 211–217, 2016.

[36] Z. M. Younossi, T. Gramlich, C. A. Matteoni, N. Boparai, and A. J. McCullough, "Nonalcoholic fatty liver disease in patients with type 2 diabetes," *Clinical Gastroenterology and Hepatology*, vol. 2, no. 3, pp. 262–265, 2004.

[37] A. M. Miller, D. L. Asquith, A. J. Hueber et al., "Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice," *Circulation Research*, vol. 107, no. 5, pp. 650–658, 2010.

[38] N. Pejnovic, I. Jeftic, N. Jovicic, N. Arsenijevic, and M. L. Lukic, "Galectin-3 and IL-33/ST2 axis roles and interplay in diet-induced steatohepatitis," *World Journal of Gastroenterology*, vol. 22, no. 44, pp. 9706–9717, 2016.

[39] Y. Gao, Y. Liu, M. Yang et al., "IL-33 treatment attenuated diet-induced hepatic steatosis but aggravated hepatic fibrosis," *Oncotarget*, vol. 7, no. 23, pp. 33649–33661, 2016.

[40] N. Jovicic, I. Jeftic, M. M. Kovacevic et al., "ST2 deficiency ameliorates high fat diet-induced liver steatosis in BALB/c mice," *Serbian Journal of Experimental and Clinical Research*, vol. 16, no. 1, pp. 9–20, 2015.

[41] B. Rehermann and M. Nascimbeni, "Immunology of hepatitis B virus and hepatitis C virus infection," *Nature Reviews Immunology*, vol. 5, no. 3, pp. 215–229, 2005.

[42] S. D. Crockett and E. B. Keeffe, "Natural history and treatment of hepatitis B virus and hepatitis C virus coinfection," *Annals of Clinical Microbiology and Antimicrobials*, vol. 4, p. 13, 2005.

[43] J. Wang, P. Zhao, H. Guo et al., "Serum IL-33 levels are associated with liver damage in patients with chronic hepatitis C," *Mediators of Inflammation*, vol. 2012, Article ID 819636, 7 pages, 2012.

[44] N. M. Hamdi, H. S. Al-Jurayb, H. M. Al-Nafea, R. A. Safar, K. M. Al Eisa, and N. M. Aref, "Evaluate the sustainability of viral response to antiviral treatment by IL33 assessment among hepatitis C virus Saudi patients," *Clinical Medicine and Diagnostics*, vol. 6, no. 6, pp. 129–136, 2016.

[45] A. H. H. Wael and A. H. Mohamed, "Hepatitis C virus pathogenesis: serum IL-33 level indicates liver damage," *African Journal of Microbiology Research*, vol. 9, no. 20, pp. 1386–1393, 2015.

[46] J. Chen and Z. Yuan, "Interplay between hepatitis B virus and the innate immune responses: implications for new therapeutic strategies," *Virologica Sinica*, vol. 29, no. 1, pp. 17–24, 2014.

[47] S. L. Huan, J. G. Zhao, Z. L. Wang, S. Gao, and K. Wang, "Relevance of serum interleukin-33 and ST2 levels and the natural course of chronic hepatitis B virus infection," *BMC Infectious Diseases*, vol. 16, p. 200, 2016.

[48] M. I. Arshad, S. Patrat-Delon, C. Piquet-Pellorce et al., "Pathogenic mouse hepatitis virus or poly(I:C) induce IL-33 in hepatocytes in murine models of hepatitis," *PLoS One*, vol. 8, no. 9, article e74278, 2013.

[49] Y. J. Liang, Z. L. Jie, L. Soong, and J. Sun, "IL-33 promotes innate IFN-γ production and controls multi-functional T cell responses in viral hepatitis (IRC7P.429)," *Journal of Immunology*, vol. 194, Supplement 1, p. 128.10, 2015.

[50] Y. J. Liang, Z. L. Jie, L. F. Hou et al., "IL-33 promotes innate IFN-γ production and modulates dendritic cell response in LCMV-induced hepatitis in mice," *European Journal of Immunology*, vol. 45, no. 11, pp. 3052–3063, 2015.

[51] Y. J. Liang, Z. L. Jie, L. F. Hou et al., "IL-33 induces nuocytes and modulates liver injury in viral hepatitis," *Journal of Immunology*, vol. 190, no. 11, pp. 5666–5675, 2013.

[52] V. Volarevic, M. Mitrovic, M. Milovanovic et al., "Protective role of IL-33/ST2 axis in Con A-induced hepatitis," *Journal of Hepatology*, vol. 56, no. 1, pp. 26–33, 2012.

[53] M. I. Arshad, M. Rauch, A. L'Helgoualc'h et al., "NKT cells are required to induce high IL-33 expression in hepatocytes during ConA-induced acute hepatitis," *European Journal of Immunology*, vol. 41, no. 8, pp. 2341–2348, 2011.

[54] M. I. Arshad, C. Piquet-Pellorce, A. L'Helgoualc'h et al., "TRAIL but not FasL and TNFα, regulates IL-33 expression in murine hepatocytes during acute hepatitis," *Hepatology*, vol. 56, no. 6, pp. 2353–2362, 2012.

[55] G. Noel, M. I. Arshad, A. Filliol et al., "Ablation of interaction between IL-33 and $ST2^+$ regulatory T cells increases immune cell-mediated hepatitis and activated NK cell liver infiltration," *American Journal of Physiology Gastrointestinal and Liver Physiology*, vol. 311, no. 2, pp. G313–G323, 2016.

[56] J. Chen, L. H. Duan, A. Xiong et al., "Blockade of IL-33 ameliorates Con A-induced hepatic injury by reducing NKT cell activation and IFN-γ production in mice," *Journal of Molecular Medicine*, vol. 90, no. 12, pp. 1505–1515, 2012.

[57] S. L. Friedman, "Liver fibrosis – from bench to bedside," *Journal of Hepatology*, vol. 38, Supplement 1, pp. S38–S53, 2003.

[58] J. P. Iredale, "Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ," *The Journal of Clinical Investigation*, vol. 117, no. 3, pp. 539–548, 2007.

[59] T. A. Wynn, "Fibrotic disease and the T_H1/T_H2 paradigm," *Nature Reviews Immunology*, vol. 4, no. 8, pp. 583–594, 2004.

[60] P. Marvie, M. Lisbonne, A. L'Helgoualc'h et al., "Interleukin-33 overexpression is associated with liver fibrosis in mice and humans," *Journal of Cellular and Molecular Medicine*, vol. 14, no. 6b, pp. 1726–1739, 2010.

[61] K. Moro, T. Yamada, M. Tanabe et al., "Innate production of T_H2 cytokines by adipose tissue-associated c-$Kit^+Sca\text{-}1^+$ lymphoid cells," *Nature*, vol. 463, no. 7280, pp. 540–544, 2010.

[62] A. E. Price, H. E. Liang, B. M. Sullivan et al., "Systemically dispersed innate IL-13-expressing cells in type 2 immunity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 25, pp. 11489–11494, 2010.

[63] T. McHedlidze, M. Waldner, S. Zopf et al., "Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis," *Immunity*, vol. 39, no. 2, pp. 357–371, 2013.

[64] Z. M. Tan, Q. H. Liu, R. Q. Jiang et al., "Interleukin-33 drives hepatic fibrosis through activation of hepatic stellate cells," *Cellular & Molecular Immunology*, vol. 14, pp. 1–11, 2017.

[65] C. Selmi, C. L. Bowlus, M. E. Gershwin, and R. L. Coppel, "Primary biliary cirrhosis," *Lancet*, vol. 377, no. 9777, pp. 1600–1609, 2011.

[66] Y. Q. Sun, J. Y. Zhang, S. Lv et al., "Interleukin-33 promotes disease progression in patients with primary biliary cirrhosis," *The Tohoku Journal of Experimental Medicine*, vol. 234, no. 4, pp. 255–261, 2014.

[67] Y. Yang, J. B. Wang, Y. M. Li et al., "Role of IL-33 expression in oncogenesis and development of human hepatocellular carcinoma," *Oncology Letters*, vol. 12, no. 1, pp. 429–436, 2016.

[68] S. M. Brunner, C. Rubner, R. Kesselring et al., "Tumor-infiltrating, interleukin-33-producing effector-memory $CD8^+$ T cells in resected hepatocellular carcinoma prolong patient survival," *Hepatology*, vol. 61, no. 6, pp. 1957–1967, 2015.

[69] D. Bergis, V. Kassis, A. Ranglack et al., "High serum levels of the interleukin-33 receptor soluble ST2 as a negative prognostic factor in hepatocellular carcinoma," *Translational Oncology*, vol. 6, no. 3, pp. 311–318, 2013.

[70] H. Peng, Q. X. Zhang, X. J. Li et al., "IL-33 contributes to *Schistosoma japonicum*-induced hepatic pathology through induction of M2 macrophages," *Scientific Reports*, vol. 6, p. 29844, 2016.

[71] O. Rostan, J. P. Gangneux, C. Piquet-Pellorce et al., "The IL-33/ST2 axis is associated with human visceral leishmaniasis and suppresses Th1 responses in the livers of BALB/c mice infected with *Leishmania donovani*," *MBio*, vol. 4, no. 5, pp. e00383–e00413, 2013.

[72] N. Sakai, H. L. Van Sweringen, R. C. Quillin et al., "Interleukin-33 is hepatoprotective during liver ischemia/reperfusion in mice," *Hepatology*, vol. 56, no. 4, pp. 1468–1478, 2012.

[73] S. Li, F. X. Zhu, H. B. Zhang, H. Li, and Y. Z. An, "Pretreatment with interleukin-33 reduces warm hepatic ischemia/reperfusion injury in mice," *Chinese Medical Journal*, vol. 126, no. 10, pp. 1855–1859, 2013.

[74] R. Weiskirchen and F. Tacke, "Interleukin-33 in the pathogenesis of liver fibrosis: alarming ILC2 and hepatic stellate cells," *Cellular & Molecular Immunology*, vol. 14, no. 2, pp. 143–145, 2017.

[75] B. Gao and R. Bataller, "Alcoholic liver disease: pathogenesis and new therapeutic targets," *Gastroenterology*, vol. 141, no. 5, pp. 1572–1585, 2011.

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Clinical Study

Pretreatment Liver Injury Predicts Poor Prognosis of DLBCL Patients

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Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous group of lymphoma, with different clinical manifestation and prognosis. The International Prognostic Index (IPI), an index designed during the prerituximab era for aggressive lymphoma, showed variable values in the prediction of patient clinical outcomes. The aim of this study was to analyze the prognostic value and causes of pretreatment liver injury in 363 de novo DLBCL patients in our institution. Pretreatment liver impairment, commonly detected in lymphoma patients, showed significant association with poor outcomes and increased serum inflammatory cytokines in DLBCL patients but had no relation to hepatitis B virus replication nor lymphomatous hepatic infiltration. Multivariate analysis revealed that liver dysfunction, advanced Ann Arbor stage, and elevated lactate dehydrogenase (LDH) were independent adverse prognostic factors of both PFS and OS. Accordingly, a new liver-IPI prognostic model was designed by adding liver injury as an important factor in determining IPI score. Based on Kaplan-Meier curves for PFS and OS, the liver-IPI showed better stratification in DLBCL patients than either the IPI or the revised IPI in survival prediction.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL) [1], while displaying great heterogeneity in clinical manifestation, disease course, and prognosis. The International Prognostic Index (IPI), based on age, performance status, lactate dehydrogenase (LDH), Ann Arbor stage, and extranodal involvements, was originally designed for prediction of prognosis in aggressive lymphoma during the prerituximab era [2]. Although already proven, in a cohort of 2031 patients, it is helpful to stratify DLBCL patients into low-, low-intermediate-, high-intermediate-, and high-risk groups, with 5-year overall survival (OS) rates of 73%, 51%, 43%, and 26%, respectively [2]. Recently, the revised IPI (R-IPI) and National Comprehensive Cancer Network IPI (NCCN-IPI) appear to better predict prognosis in DLBCL patients. The R-IPI identifies three distinct prognostic groups with outcomes categorized as very good (patients with no IPI risk factors, 4-year OS 94%), good (patients with 1 or 2 risk factors, 4-year OS 79%), and poor (patients with 3–5 risk factors, 4-year OS 55%), respectively [3]. The NCCN-IPI is based on five predictors (age, LDH, extranodal sites, Ann Arbor stage, and performance status) and 4 prognostic groups (low (score 0-1), low-intermediate (score 2-3), high-intermediate (score 4-5), and high (score 6–8)). The NCCN-IPI better separates

low- and high-risk subgroups (5-year OS: 96% versus 33%, resp.) than the IPI (5-year OS: 90% versus 54%, resp.) [4].

Cytokines are documented to be closely associated with both inflammation and immune modulation while playing a key role in the development of liver damage in a variety of liver disease such as chronic hepatitis B virus (HBV) infection, alcoholic liver injury, nonalcoholic fatty liver disease, and drug-induced liver injury [5–8]. It is generally believed that cytokines are deregulated in many kinds of haematological disorders [9, 10], while elevation of interleukin- (IL-) 6, IL-10, tumor necrosis factor- (TNF-) α, IL-8, and IL-2 receptor (IL-2R) was demonstrated valuable in the prediction of unfavorable prognosis in lymphoma [11–14].

The aim of the present study was to determine the role of liver inflammation, reflected by the cytokines and serum transaminase activities, gamma-glutamyltranspeptidase (γ-GT), and alkaline phosphatase (ALP) in the prediction of outcome in DLBCL patients.

2. Patients and Methods

2.1. Patients. We conducted a single-center retrospective case-control study on de novo DLBCL patients. A total of 363 patients were included, with the histological classification confirmed according to the World Health Organization (WHO) 2008 criteria [15]. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-GT, and ALP were used as markers of liver injury as recommended by the regulatory authorities [16]. Serum cytokine tests (including IL-1β, IL-2R, IL-6, IL-8, IL-10, and TNF-α) were systematically assessed before chemotherapy. Cytokines were detected in the serum of 15 healthy volunteers as controls. Clinical characteristics of the 363 patients are shown in Table 1. Patients with abnormal liver function, defined as elevation in any of the following four indexes: ALT, AST, γ-GT, or ALP, were recruited into the liver dysfunction group; then, a propensity score matching method was used to create the matched control group [17]. Patients were matched at a ratio of 1 : 1 using the nearest neighbor method with a caliber of 0.10. All the patients and volunteers gave their informed consent, following the regulations of the Shanghai Jiao Tong University School of Medicine Institutional Review Boards, in accordance with the Declaration of Helsinki.

2.2. Treatment Regimens. 340 patients (93.7%) received R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), and 15 patients (4.1%) received CHOP chemotherapy as initial treatment. The rest 8 patients (2.2%) received only palliative care in consideration of the poor performance status or insufficient organ function (Table 1).

2.3. Response Criteria. The treatment response was evaluated according to the WHO response criteria [18]. Complete response (CR) was defined as no evidence of residual disease, partial response (PR) as having at least a 50% reduction in tumor burden from the onset of treatment, and no response as having less than a 50% reduction in tumor burden or disease progression. Assessment of the treatment response was evaluated by a follow-up clinical, radiological, or laboratory study, as determined by the clinician, as described previously [19, 20].

2.4. Statistical Analysis. Baseline characteristics of patients were analyzed using Student's t-tests for continuous variables, χ^2 tests for categorical data, and Mann–Whitney U test for the serum level of cytokines. Overall survival (OS) time was measured from the date of diagnosis to the date of death or to the last follow-up. Progression-free survival (PFS) was calculated from the date when the treatment began to the date when the disease progression was recognized or the date of the last follow-up as described previously [19, 20]. Survival functions were estimated using the Kaplan-Meier method and compared by the log-rank test. Univariate hazard estimates were generated with unadjusted Cox proportional hazards. Multivariate survival analysis was performed using a Cox regression model in which significant variables in the univariate analysis were included. $p < 0.05$ was considered statistically significant. All statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) 22.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Liver Dysfunction in De Novo DLBCL Patients Was Associated with Poor Outcome and High Cytokine Levels in the Serum. Transaminase activities, γ-GT, and ALP were measured before chemotherapy in 363 de novo DLBCL patients. Liver injury was observed in 87 patients. The median values of ALT, AST, γ-GT, and ALP for those patients with liver dysfunction were 41.0 IU/L (range 10.0 to 577.0), 45.0 IU/L (range 7.0 to 678.0), 65.5 IU/L (range 1.0 to 707.0), and 89.0 IU/L (range 21.0 to 1013.0), respectively, significantly higher than those for the 276 patients without liver dysfunction ($p < 0.001$, Table 1). Patients with liver dysfunction had no relationship with HBV replication or lymphomatous hepatic infiltration but were significantly associated with advanced Ann Arbor stage ($p < 0.001$), poor performance status ($p < 0.001$), increased LDH level ($p < 0.001$), high IPI score ($p < 0.001$), presence of B symptoms ($p = 0.002$), and low CR rate ($p = 0.004$, Table 1). Since cytokines in the serum had been reported to be associated with liver inflammation and dysfunction [5–8], patients with liver dysfunction had significantly higher level of IL-2R, IL-6, IL-10, and TNF-α in the serum, when compared with those without liver dysfunction ($p < 0.001$, Table 1).

3.2. Liver Dysfunction Was Associated with Poor Outcome and High Serum Cytokine Levels in Matched Case-Control Analysis in DLBCL. To avoid the unfavorable impact of advanced disease stage and high IPI score on the outcome of the patients, 87 of 276 patients without liver dysfunction were selected as case controls using 1 : 1 matching on propensity scores for sex, age, IPI score, and lymphomatous hepatic infiltration, with a caliber of 0.10. Clinical characteristics of the 174 patients selected by propensity score matching are shown in Supplement Table 1S available online at https://doi.org/10.1155/2017/7960907. After matching,

Table 1: Clinical characteristics of DLBCL patients ($n = 363$).

Characteristics	Liver dysfunction group, n (%)	Normal liver function group, n (%)	p value
Average age (years)	56.7	55.8	0.760
Age (years) > 60	35 (40%)	140 (51%)	0.088
Sex (male)	56 (64%)	156 (57%)	0.195
IPI score			<0.001
Low	26 (30%)	147 (53%)	
Low-intermediate	17 (20%)	54 (19%)	
High-intermediate	20 (23%)	43 (16%)	
High	24 (27%)	32 (12%)	
Ann Arbor stages III-IV	57 (66%)	104 (38%)	<0.001
Number of extranodal sites ≥ 2	35 (40%)	90 (33%)	0.192
Lymphomatous hepatic infiltration	7 (8%)	9 (3%)	0.058
LDH > normal	60 (69%)	94 (34%)	<0.001
Performance status (ECOG) ≥ 2	25 (29%)	27 (10%)	<0.001
Presence of B symptoms	33 (38%)	59 (21%)	0.002
HBV-DNA positive	5 (6%)	8 (3%)	0.213
Hepatitis C virus	1 (1%)	4 (1%)	0.655
Liver enzyme (median values [range], IU/L)			
ALT	41.0 (10.0–577.0)	16.5 (1.0–59.0)	<0.001
AST	45.0 (7.0–678.0)	19.0 (9.0–39.0)	<0.001
γ-GT	65.5 (1.0–707.0)	18.0 (1.0–64.0)	<0.001
ALP	89.0 (21.0–1013.0)	69.0 (39.0–122.0)	<0.001
Serum cytokines (median values [range])			
IL-2R (U/mL)	1894.5 (232.0–7500.0)	615.5 (52.1–7500.0)	<0.001
IL-6 (pg/mL)	8.9 (2.0–194.0)	3.6 (2.0–69.1)	<0.001
IL-8 (pg/mL)	43.7 (6.6–3533.0)	54.0 (5.0–2849.0)	0.207
IL-10 (pg/mL)	7.1 (5.0–1000.0)	5.0 (4.0–1000.0)	<0.001
TNF-α (pg/mL)	19.2 (4.0–275.0)	9.5 (4.0–151.0)	<0.001
Treatment			<0.001
R-CHOP	75 (86%)	265 (96%)	
CHOP	4 (5%)	11 (4%)	
Supportive care	8 (9%)	0 (0%)	
CR (%)	70.0	85.8	0.004

elevated LDH level was still observed in patients with liver dysfunction (Table 1S).

With a median follow-up of 11.7 months in both groups, patients in the liver dysfunction group showed significantly poorer outcomes than those in the matched control group (liver dysfunction group: 2-year PFS 58.5% and 2-year OS 65.2%; matched control group: 2-year PFS 74.0% and 2-year OS 84.9%, $p = 0.019$ and 0.001, resp.; Figure 1(a)).

In subgroup analysis according to IPI score, in patients with IPI score 0–2, no significant difference was found for PFS or OS between the matched control group and liver dysfunction group ($p = 0.657$ and $p = 0.156$, resp.; Figure 1(b)). However, in patients with IPI score 3–5, patients in the liver dysfunction group showed significantly shorter PFS and OS when compared with those in the matched control group ($p < 0.001$ and $p = 0.002$, resp.; Figure 1(c)). Of note, patients in the liver dysfunction group retained significantly higher levels of serum cytokines IL-2R, IL-6, IL-10, and TNF-α, compared with those in the matched control group ($p = 0.003$, $p = 0.022$, $p = 0.045$, and $p < 0.001$, resp.; Figure 2 and Table 1S) and healthy volunteers (all $p < 0.001$; Figure 2). Interestingly, patients in the matched control group, compared with healthy volunteers, also showed significantly higher serum levels of IL-2R, IL-6, IL-10, and TNF-α ($p < 0.001$, $p < 0.001$, $p = 0.015$, and $p < 0.001$, resp.; Figure 2).

3.3. Liver Dysfunction Was an Independent Adverse Prognostic Factor by Univariate and Multivariate Analyses in DLBCL. As shown in Table 2, in univariate analysis, decreased OS and PFS rates correlated with high IPI score (both $p < 0.001$), advanced Ann Arbor stage (both $p < 0.001$), poor performance status (both $p < 0.001$), and elevated LDH level (both $p < 0.001$) as well as cytokines IL-2R (both $p < 0.001$), IL-6 ($p < 0.001$ and $p = 0.004$, resp.), IL-10 (both $p < 0.001$), and TNF-α ($p = 0.003$ and $p = 0.005$, resp.).

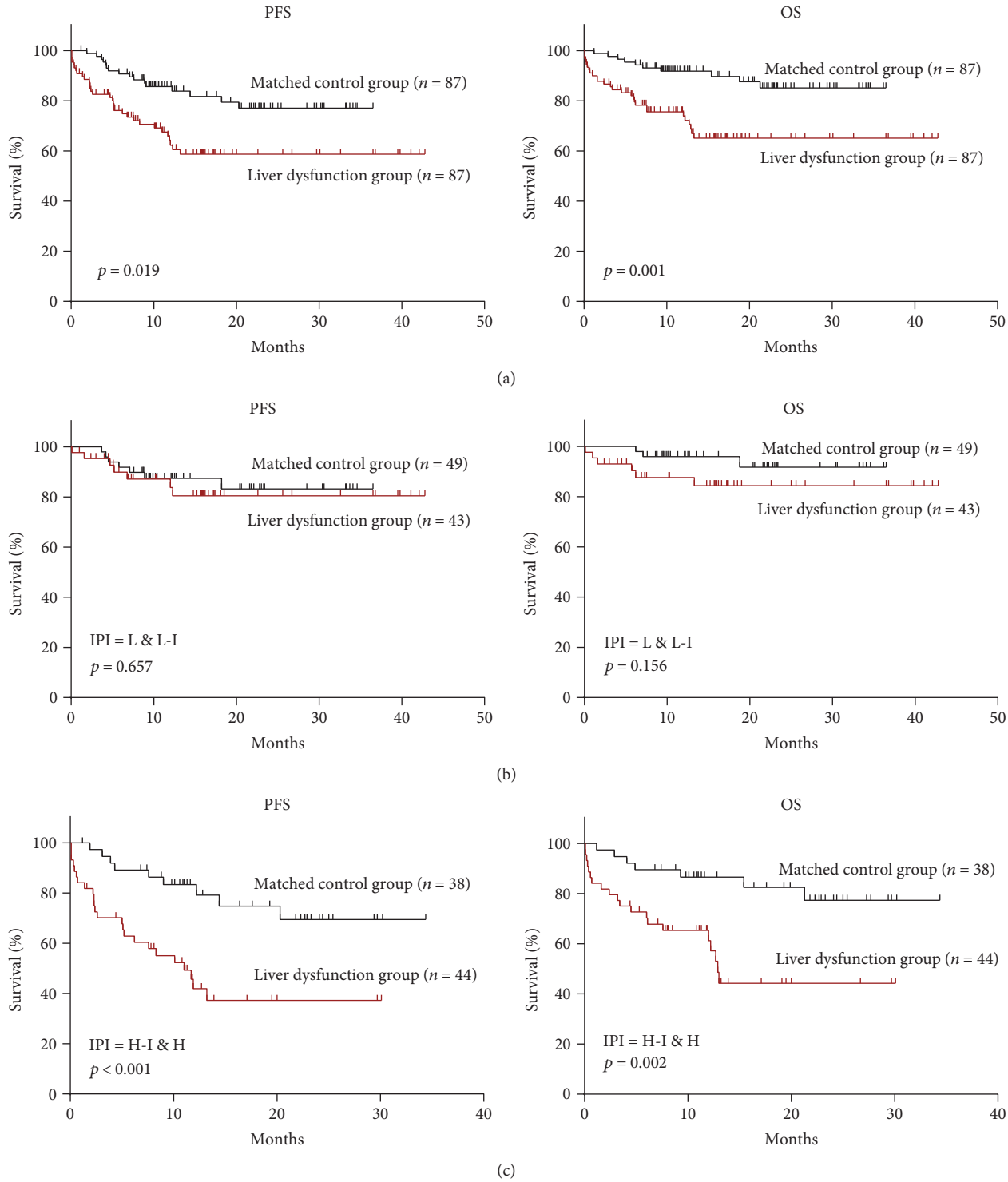

Figure 1: Progression-free survival (PFS) and overall survival (OS) curves based on pretreatment liver function in (a) 174 patients selected by propensity score matching, (b) International Prognostic Index (IPI) low- (L-) and low-intermediate- (L-I-) risk patients, and (c) IPI high-intermediate- (H-I-) and high- (H-) risk patients.

Importantly, liver dysfunction was strongly associated with shorter PFS and OS (both $p < 0.001$). Multiple extranodal involvement was of prognostic value only for PFS ($p = 0.019$), and the presence of B symptoms was of prognostic value only for OS ($p = 0.036$).

In multivariate analysis, after incorporating all variables that were significant in univariate analysis, elevated ALT, AST, γ-GT, or ALP levels (OR = 1.815, 95% CI 1.075–3.064, $p = 0.026$); advanced Ann Arbor stage (OR = 4.013, 95% CI 2.073–7.769, $p < 0.001$), elevated LDH level (OR = 2.460,

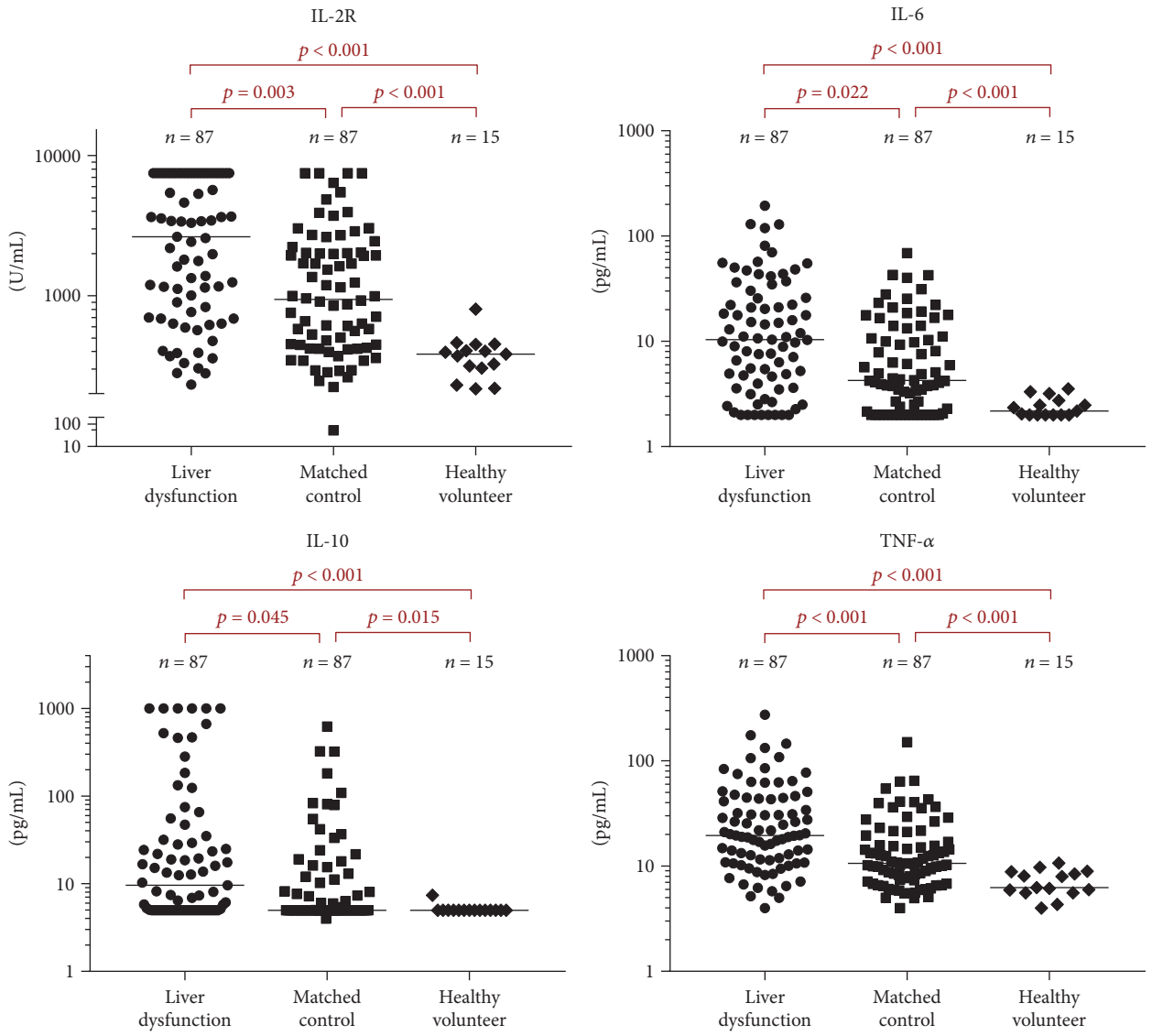

Figure 2: Serum interleukin- (IL-) 2 receptor (IL-2R), IL-6, IL-10, and tumor necrosis factor- (TNF-) α levels in the liver dysfunction group, matched control group, and healthy volunteers.

95% CI 1.350–4.482, $p = 0.003$); and IL-6 (OR = 2.460, 95% CI 1.142–5.299, $p = 0.022$) predicted shorter PFS. Similarly, liver dysfunction (OR = 3.352, 95% CI 1.730–6.496, $p < 0.001$), advanced Ann Arbor stage (OR = 3.194, 95% CI 1.435–7.110, $p = 0.004$), and elevated LDH level (OR = 4.404, 95% CI 1.871–10.366, $p < 0.001$) retained their independent prognostic impact on shorter OS (Table 3).

3.4. The New Prognostic Model Liver-IPI Was Developed in Our DLBCL Cohort. Since liver dysfunction is an independent prognostic factor for both PFS and OS, it was combined with the IPI to design a new prognostic model, named as the liver-IPI. In the liver-IPI model, elevation of ALT, AST, γ-GT, or ALP was scored as 1 point, combined with IPI 5 scores to reach a total score of 6. Three risk groups were formed: low-risk (0-1 scores), intermediate-risk (2-3 scores), and high-risk (4–6 scores). The liver-IPI showed better stratification of patients than either the IPI or the R-IPI in OS and PFS, since significant differences were found between low- and intermediate-risk groups (PFS ($p < 0.001$) and OS ($p = 0.016$); Figure 3(c)), as well as in intermediate- versus high-risk groups ($p < 0.001$ for both PFS and OS; Figure 3(c)). However, according to the IPI, no significant difference of OS and PFS was found between the low-intermediate-risk group and high-intermediate-risk group ($p = 0.251$ and $p = 0.443$, resp.; Figure 3(a)). Similarly, no difference of PFS was found between high-intermediate- and high-risk groups ($p = 0.058$; Figure 3(a)). For the R-IPI, there was no statistic difference of OS between the very good and good groups ($p = 0.114$; Figure 3(b)).

4. Discussion

To our knowledge, this is the first report showing that pretreatment liver dysfunction was associated with poor prognosis in patients with DLBCL. Elevated serum

Table 2: Univariate analyses on PFS and OS in DLBCL patients ($n = 363$).

Variates	2-year PFS rate (%)	p value for PFS	2-year OS rate (%)	p value for OS
IPI score		<0.001		<0.001
Low	91.6		94.9	
Low-intermediate	68.1		85.0	
High-intermediate	61.7		72.1	
High	40.7		50.7	
Ann Arbor stage		<0.001		<0.001
I-II	90.9		93.9	
III-IV	54.3		68.3	
Number of extranodal sites		0.019		0.176
≤1	80.0		85.6	
≥2	62.9		75.6	
Performance status (ECOG)		<0.001		<0.001
≤1	77.8		86.3	
≥2	54.2		60.0	
LDH		<0.001		<0.001
Normal	87.7		94.4	
>Normal	55.0		65.1	
Liver enzyme		<0.001		<0.001
Normal	79.8		88.0	
>Normal	59.5		65.2	
IL-2R		<0.001		<0.001
Normal	90.9		95.1	
>Normal	63.2		74.9	
IL-6		<0.001		0.004
Normal	88.2		91.1	
>Normal	69.2		80.1	
IL-10		<0.001		<0.001
Normal	80.7		87.9	
>Normal	64.0		74.5	
TNF-α		0.003		0.005
Normal	88.0		96.5	
>Normal	68.8		76.9	
B symptoms		0.065		0.036
Present	78.0		85.8	
Absent	63.9		72.1	

transaminase activities, γ-GT, and ALP were significantly associated with extended lymphoma disease (advanced Ann Arbor stage, elevated LDH level) and alteration of the host status (poor performance status and presence of B symptoms). Meanwhile, it is also revealed that impaired liver function is not directly caused by HBV replication or lymphomatous hepatic infiltration. Of note, in the liver dysfunction group, significant poor treatment outcome with shorter PFS and OS was observed, particularly in those patients of high-intermediate and high risk.

Furthermore, in multivariate Cox regression analysis, pretreatment liver function impairment was an independent unfavorable prognostic factor, which fully demonstrated the prognostic value of liver injury on DLBCL. Therefore, a new prognostic model based on liver function and IPI score, liver-IPI, was designed. The liver-IPI showed a better stratification of different outcomes in patients than the IPI and R-IPI.

In the liver dysfunction group, patients had significantly higher level of IL-2R, IL-6, IL-10, and TNF-α, when compared with those in the normal liver function group. Accumulating data has shown that an imbalance in cytokine production is critically involved in the development of liver damage in a variety of liver diseases. TNF-α, a central regulator of inflammatory and immune responses, is secreted by activated monocytes, macrophages, and T lymphocytes [21, 22]. Increased TNF-α production not only contributes to chronic alcoholic liver injury [23] but also influences the nonalcoholic fatty liver disease process [7]. Soluble IL-2R (sIL-2R) is the soluble form of IL-2R, which is expressed on the cell membrane of lymphocytes and plays an important role in their activation and proliferation [24]. It is released from activated T-cells mainly due to the cleavage by proteinase matrix metalloproteinase-9 produced by inflammation-related cells [25]. The level of sIL-2R reflects the extent of inflammation [26] and correlate with fibrosis stages in patients with chronic HBV infection [5]. Increased IL-6 and IL-10, two major inflammatory cytokines, are reported in ethanol-induced hepatocellular damage and concanavalin A-induced liver injury [27]. *In vivo*, cytokines usually form a network to augment the inflammation and liver impairment. As a mechanism of action, following the induction of IL-6, IL-8, and IL-10 secretion, TNF-α could activate the nuclear factor-kappa B pathway and enhance the adhesion molecule expression, which in turn results in adherence of neutrophils and monocytes to the endothelium. Accumulation and activation of inflammatory cells further generate ROS and NO and induce liver damage [8, 28–31]. These mechanisms partially explained the phenomena that pretreatment liver injury was associated with high level of cytokines and poor outcome of patients, without being related to the HBV replication and lymphomatous hepatic infiltration.

Univariate analysis revealed that elevated serum cytokines IL-2R, IL-6, IL-10, and TNF-α correlated with the decreased OS and PFS rate. Accumulating researches have pointed out that in lymphoma patients, TNF-α accumulation is associated with lymphoma progression [32] and serum sIL-2R is a predictor of poor outcome in DLBCL patients [13, 33]. IL-6 and IL-10 belong to T-helper type 2 cell cytokines, contributing to inhibition of host's immune system and induction of tumor progression [34, 35]. Several studies showed that increased levels of serum IL-6 and IL-10 indicated a poor therapeutic response rate and short survival time in DLBCL [11, 12, 36–38].

5. Conclusion

Pretreatment liver injury was an independent poor prognostic factor in newly diagnosed DLBCL patients, correlating with increased serum levels of liver dysfunction-associated

Table 3: Multivariate analyses on PFS and OS in DLBCL patients ($n = 363$).

Variates	PFS	95% CI	p value	OS	95% CI	p value
Liver dysfunction	1.815	1.075–3.064	0.026	3.352	1.730–6.496	<0.001
Ann Arbor stages III-IV	4.013	2.073–7.769	<0.001	3.194	1.435–7.110	0.004
LDH	2.460	1.350–4.482	0.003	4.404	1.871–10.366	<0.001
IL-6	2.460	1.142–5.299	0.022			

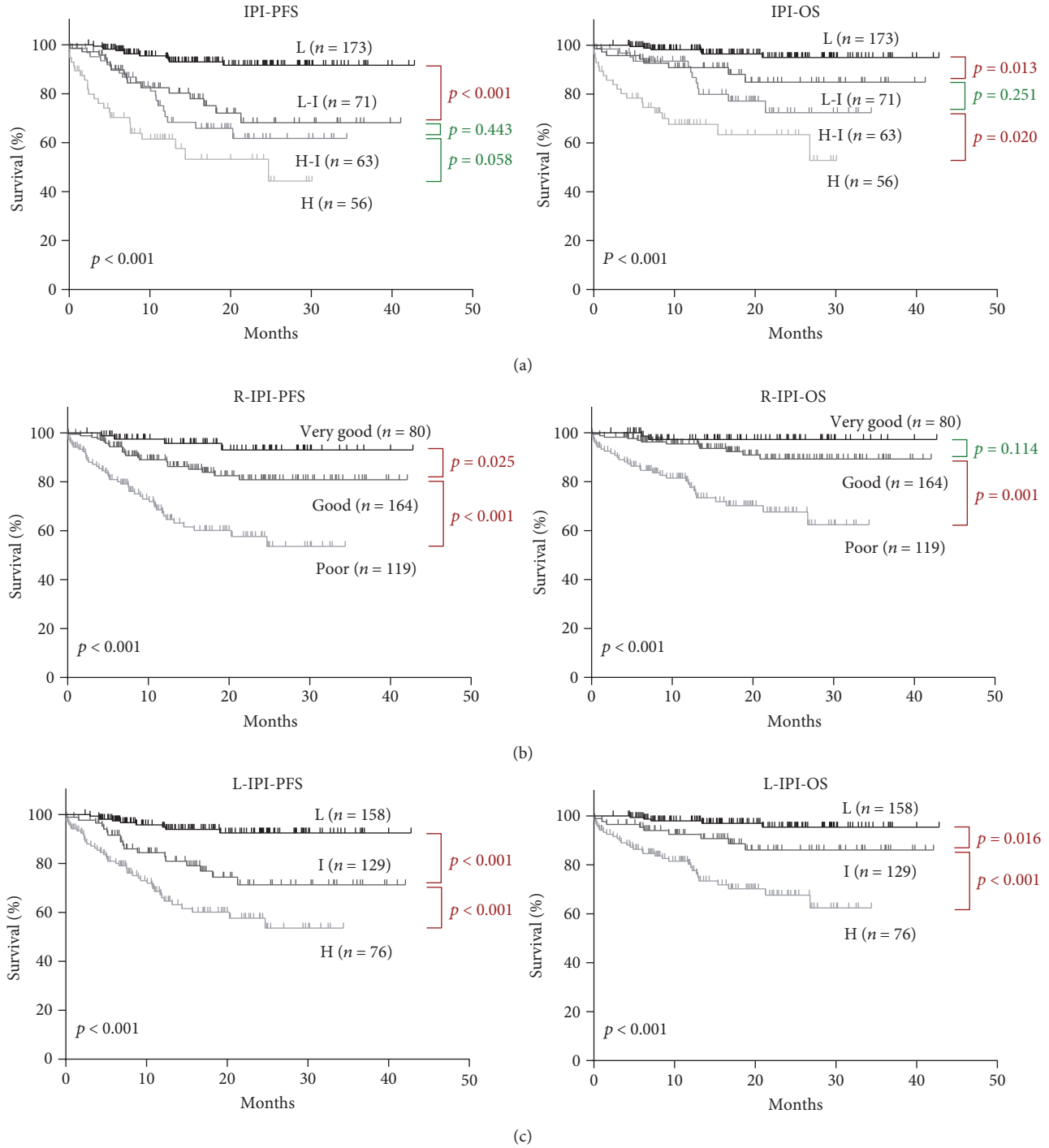

Figure 3: Progression-free survival (PFS) and overall survival (OS) curves according to (a) the International Prognostic Index (IPI), (b) the revised IPI (R-IPI), and (c) the liver-IPI (L-IPI). Four risk groups for IPI score: low- (L-), low-intermediate- (L-I-), high-intermediate- (H-I-), and high- (H-) risk groups. Three risk groups for L-IPI score: low- (L-), intermediate- (I-), and high- (H-) risk groups.

cytokines IL-2R, IL-6, IL-10, and TNF-α. In addition, liver-IPI, based on liver function and IPI score, had a satisfactory prognostic value in the risk stratification of DLBCL.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Wei-Li Zhao, Li Wang, Anne Janin, and Christophe Leboeuf designed the research study; Chao-Fu Wang and Bin-Shen Ou-Yang performed the pathological analysis; Qing Shi, Rong Shen, Ying Qian collected the data; Xing Fan, Yan Zhao, Shu Cheng provided the patients; Christophe Leboeuf analyzed the data; and Wei-Li Zhao and Li Wang wrote the paper. All authors approved the final manuscript. Qing Shi, Rong Shen, Chao-Fu Wang, and Xing Fan contributed equally to this work.

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References

[1] N. Listed, "A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The non-Hodgkin's lymphoma classification project," *Blood*, vol. 89, no. 11, p. 3909, 1997.

[2] The International Non-Hodgkin's Lymphoma Prognostic Factors Project, "A predictive model for aggressive non-Hodgkin's lymphoma," *The New England Journal of Medicine*, vol. 329, no. 14, pp. 987–994, 1993.

[3] L. H. Sehn, B. Berry, M. Chhanabhai et al., "The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP," *Blood*, vol. 109, no. 5, pp. 1857–1861, 2007.

[4] Z. Zhou, L. H. Sehn, A. W. Rademaker et al., "An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era," *Blood*, vol. 123, no. 6, pp. 837–842, 2014.

[5] Y. Q. Deng, H. Zhao, A. L. Ma et al., "Selected cytokines serve as potential biomarkers for predicting liver inflammation and fibrosis in chronic hepatitis B patients with normal to mildly elevated aminotransferases," *Medicine*, vol. 94, no. 45, article e2003, 2015.

[6] H. Kawaratani, T. Tsujimoto, A. Douhara et al., "The effect of inflammatory cytokines in alcoholic liver disease," *Mediators of Inflammation*, vol. 2013, Article ID 495156, 10 pages, 2013.

[7] V. Braunersreuther, G. L. Viviani, F. Mach, and F. Montecucco, "Role of cytokines and chemokines in non-alcoholic fatty liver disease," *World Journal of Gastroenterology*, vol. 18, no. 8, pp. 727–735, 2012.

[8] S. Lacour, J. C. Gautier, M. Pallardy, and R. Roberts, "Cytokines as potential biomarkers of liver toxicity," *Cancer Biomarkers*, vol. 1, no. 1, pp. 29–39, 2005.

[9] R. Kurzrock, "Cytokine deregulation in cancer," *Biomedicine & Pharmacotherapy*, vol. 55, no. 9-10, pp. 543–547, 2001.

[10] G. Salles and B. Coiffier, "Inherited cytokine response and risk of lymphoma," *Lancet Oncology*, vol. 7, no. 1, pp. 3-4, 2006.

[11] A. Nacinovic-Duletic, S. Stifter, S. Dvornik, Z. Skunca, and N. Jonjić, "Correlation of serum IL-6, IL-8 and IL-10 levels with clinicopathological features and prognosis in patients with diffuse large B-cell lymphoma," *International Journal of Laboratory Hematology*, vol. 30, no. 3, pp. 230–239, 2008.

[12] A. Duletic-Nacinovic, S. Stifter, B. Marijic et al., "Serum IL-6, IL-8, IL-10 and beta2-microglobulin in association with International Prognostic Index in diffuse large B cell lymphoma," *Tumori*, vol. 94, no. 4, pp. 511–517, 2008.

[13] N. Goto, H. Tsurumi, H. Goto et al., "Serum soluble interleukin-2 receptor (sIL-2R) level is associated with the outcome of patients with diffuse large B cell lymphoma treated with R-CHOP regimens," *Annals of Hematology*, vol. 91, no. 5, pp. 705–714, 2012.

[14] E. Lech-Maranda, J. Bienvenu, F. Broussais-Guillaumot et al., "Plasma TNF-α and IL-10 level-based prognostic model predicts outcome of patients with diffuse large B-cell lymphoma in different risk groups defined by the International Prognostic Index," *Archivum Immunologiae et Therapiae Experimentalis (Warsz)*, vol. 58, no. 2, pp. 131–141, 2010.

[15] S. H. Swerdlow, E. Campo, N. L. Harris, E. S. Jaffe, S. A. Pileri, H. Stein, and J. Thiele, Eds., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, IARC, Lyon, France, 2008.

[16] FDA, "Guidance for industry drug-induced liver injury: premarketing clinical evaluation," *Drug Safety*, 2007, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.

[17] R. H. Dehejia and S. Wahba, "Propensity score-matching methods for nonexperimental causal studies," *The Review of Economics and Statistics*, vol. 84, no. 1, pp. 151–161, 2002.

[18] B. D. Cheson, S. J. Horning, B. Coiffier et al., "Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group," *Journal of Clinical Oncology*, vol. 17, no. 4, p. 1244, 1999.

[19] P. Xu, D. Yu, L. Wang, Y. Shen, Z. Shen, and W. Zhao, "Analysis of prognostic factors and comparison of prognostic scores in peripheral T cell lymphoma, not otherwise specified: a single-institution study of 105 Chinese patients," *Annals of Hematology*, vol. 94, no. 2, pp. 239–247, 2015.

[20] P. P. Xu, Y. Wang, Y. Shen, L. Wang, Z. X. Shen, and W. L. Zhao, "Prognostic factors of Chinese patients with T/NK-cell lymphoma: a single institution study of 170 patients," *Medical Oncology*, vol. 29, no. 3, pp. 2176–2182, 2012.

[21] B. Ryffel, M. Brockhaus, U. Durmuller, and F. Gudat, "Tumor necrosis factor receptors in lymphoid tissues and

lymphomas. Source and site of action of tumor necrosis factor alpha," *The American Journal of Pathology*, vol. 139, no. 1, pp. 7–15, 1991.

[22] S. S. Sung, J. M. Bjorndahl, C. Y. Wang, H. T. Kao, and S. M. Fu, "Production of tumor necrosis factor/cachectin by human T cell lines and peripheral blood T lymphocytes stimulated by phorbol myristate acetate and anti-CD3 antibody," *The Journal of Experimental Medicine*, vol. 167, no. 3, pp. 937–953, 1988.

[23] Y. Iimuro, R. M. Gallucci, M. I. Luster, H. Kono, and R. G. Thurman, "Antibodies to tumor necrosis factor alfa attenuate hepatic necrosis and inflammation caused by chronic exposure to ethanol in the rat," *Hepatology*, vol. 26, no. 6, pp. 1530–1537, 2010.

[24] L. A. Rubin and D. L. Nelson, "The soluble interleukin-2 receptor: biology, function, and clinical application," *Annals of Internal Medicine*, vol. 113, no. 8, pp. 619–627, 1990.

[25] N. Yoshida, M. Oda, Y. Kuroda et al., "Clinical significance of sIL-2R levels in B-cell lymphomas," *PLoS One*, vol. 8, no. 11, article e78730, 2013.

[26] K. Poovorawan, P. Tangkijvanich, C. Chirathaworn et al., "Circulating cytokines and histological liver damage in chronic hepatitis B infection," *Hepatitis Research & Treatment*, vol. 2013, article 757246, 7 pages, 2013.

[27] B. Gao, "Hepatoprotective and anti-inflammatory cytokines in alcoholic liver disease," *Journal of Gastroenterology and Hepatology*, vol. 27, Supplement 2, pp. 89–93, 2012.

[28] S. F. Liu, X. Ye, and A. B. Malik, "Inhibition of NF-κB activation by pyrrolidine dithiocarbamate prevents in vivo expression of proinflammatory genes," *Circulation*, vol. 100, no. 12, pp. 1330–1337, 1999.

[29] S. Sethu and A. J. Melendez, "New developments on the TNF-alpha-mediated signalling pathways," *Bioscience Reports*, vol. 31, no. 1, pp. 63–76, 2011.

[30] H. Jaeschke, G. J. Gores, A. I. Cederbaum, J. A. Hinson, D. Pessayre, and J. J. Lemasters, "Mechanisms of hepatotoxicity," *Toxicological Sciences*, vol. 65, no. 2, pp. 166–176, 2002.

[31] Z. Zhou, M. C. Connell, and D. J. Macewan, "TNFR1-induced NF-κB, but not ERK, p38MAPK or JNK activation, mediates TNF-induced ICAM-1 and VCAM-1 expression on endothelial cells," *Cellular Signalling*, vol. 19, no. 6, pp. 1238–1248, 2007.

[32] G. Salles, J. Bienvenu, Y. Bastion et al., "Elevated circulating levels of TNF-α and its p55 soluble receptor are associated with an adverse prognosis in lymphoma patients," *British Journal of Haematology*, vol. 93, no. 2, pp. 352–359, 1996.

[33] Y. Oki, H. Kato, K. Matsuo et al., "Prognostic value of serum soluble interleukin-2 receptor level in patients with diffuse large B cell lymphoma, treated with CHOP- or RCHOP-based therapy," *Leukemia & Lymphoma*, vol. 49, no. 7, p. 1345, 2008.

[34] K. W. Moore, M. R. Waalde, R. L. Coffman, and A. O'Garra, "Interleukin-10 and the interleukin-10 receptor," *Annual Review of Immunology*, vol. 19, pp. 683–765, 2001.

[35] S. Mocellin, F. Marincola, C. R. Rossi, D. Nitti, and M. Lise, "The multifaceted relationship between IL-10 and adaptive immunity: putting together the pieces of a puzzle," *Cytokine & Growth Factor Reviews*, vol. 15, no. 1, pp. 61–76, 2004.

[36] H. A. Preti, F. Cabanillas, M. Talpaz, S. L. Tucker, J. F. Seymour, and R. Kurzrock, "Prognostic value of serum interleukin-6 in diffuse large-cell lymphoma," *Annals of Internal Medicine*, vol. 127, no. 3, pp. 186–194, 1997.

[37] A. Duletic-Nacinovic, M. Sever-Prebelic, S. Stifter, N. Jonjić, M. Hasan, and B. Labar, "Interleukin-6 in patients with aggressive and indolent non-Hodgkin's lymphoma: a predictor of prognosis?," *Clinical Oncology (Royal College of Radiologists)*, vol. 18, no. 4, pp. 367-368, 2006.

[38] T. H. Uskudar, E. Gunduz, O. M. Akay, C. Bal, and Z. Gülbaş, "Are the high serum interleukin-6 and vascular endothelial growth factor levels useful prognostic markers in aggressive non-Hodgkin lymphoma patients?," *Turkish Journal of Haematology*, vol. 32, no. 1, pp. 21–28, 2015.

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Review Article

Liver Regeneration: Analysis of the Main Relevant Signaling Molecules

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Liver regeneration is a highly organized tissue regrowth process and is the most important reaction of the liver to injury. The overall process of liver regeneration includes three phases: priming stage, proliferative phase, and termination phase. The initial step aims to induce hepatocytes to be sensitive to growth factors with the aid of some cytokines, including TNF-α and IL-6. The proliferation phase promotes hepatocytes to re-enter G1 with the stimulation of growth factors. While during the termination stage, hepatocytes will discontinue to proliferate to maintain normal liver mass and function. Except for cytokine- and growth factor-mediated pathways involved in regulating liver regeneration, new substances and technologies emerge to influence the regenerative process. Here, we reviewed novel and important signaling molecules involved in the process of liver regeneration to provide a cue for further research.

1. Introduction

The liver, composed of parenchymal cells—hepatocytes—and nonparenchymal cells including endothelial cells, Kupffer cells, lymphocytes, and stellate cells, has a unique capacity to precisely regulate its growth and mass, which is particularly remarkable since hepatocytes are stable cells and rarely divide in the normal state, as they are quiescent in the G0 phase of the cell cycle [1]. However, their proliferative capacity is initiated in the case of liver tissue loss. There are two different regenerative models. Partial hepatectomy (two-thirds of the liver is removed) initiates a unique response, during which the remaining diploid hepatocytes enter into the cell cycle to compensate for the loss of liver tissue, taking about a week [2]. Another pattern of the regenerative model is established by insult, such as toxins and viral infection, during which all hepatocytes are hurt and oval cells are considered as potent stem cells to differentiate into hepatocytes and biliary cells. Both of the two patterns of liver regeneration will be involved in the review.

Findings of past several decades have revealed that liver regeneration is a complex network regulated by various growth factors and cytokines expressed at the site of injury or migrated to the liver via the circulatory system. To sum it up, the regenerative process includes three critical steps [3]: firstly, quiescent hepatocytes convert from G0 to G1 of the cell cycle when faced with multiple stimulations (the priming phase); secondly, with the help of mitogens, hepatocytes progress beyond the restriction point to the G1 phase and then the mitosis (the proliferation phase); and then the last, cells terminate proliferation under the control of negative factors (the termination phase), such as transforming growth factor beta (TGF-β) and activin (Figure 1). In these three phases, various cytokines or growth factors exhibit a pivotal role through cell signaling pathways of multiple biological effects. Here, we endeavor to summarize some classical and novel signaling molecules participating in the process.

2. The Priming Phase: The Primary Molecules Tumor Necrosis Factor-α (TNF-α) and IL-6

Inflammation is a complex biological response and is characterized by recruitment, proliferation, and activation of a series of inflammatory cells and immune cells, and it aims to alleviate infections, eliminate damaged cells, and initiate tissue repair and regeneration [4]. Inflammation goes

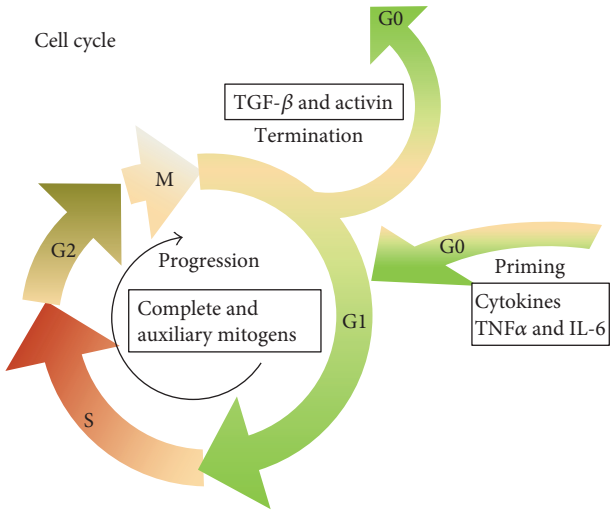

Figure 1: The outline of liver regeneration process.

through the whole process of liver damage and promotes regeneration of the injured liver. Inflammation-induced regeneration primarily is triggered by cytokines and growth factors released from inflammatory cells. The most widely studied proinflammatory cytokines are TNF-α and IL-6.

Kupffer cells are known to produce a group of cytokines and immunomodulating mediators that have stimulatory and inhibitory effects on hepatic injury. Hepatic macrophages are the main source of TNF-α and IL-6 through the NF-κB signaling pathway triggered either by Gut-derived factor lipopolysaccharide (LPS)/Toll-like receptor4 (TLR4) signaling or by C3a and C5a, components of the complement system (Figure 2). They prime hepatocytes to re-enter into the cell cycle in the first stage. Loss of either TNF-α or IL-6 could delay liver regeneration [5]. TLR4 recognizes its ligand LPS and then recruits and activates myeloid differentiation factor 88 (MyD88), triggering signal transduction downstream to promote the release of proinflammatory factors. In the view of C3 and C5, part of innate immune response which works in the process of liver injury to fight with multiple pathogens, they exert their effects on hepatocyte proliferation by activation of the bioactive peptides C3a and C5a with the stimulation of LPS [6] (Figure 2). C3a not only mediates signals to the downstream C5a but also affects hepatocyte proliferation in a C5-independent fashion [7]. Mice deficient of either C3 or C5 showed impaired liver regeneration [8]. A complement inhibitor, CR2-CD59, targeting the site of complement activation and specially inhibiting the membrane attack complex (MAC), was used to study the complement-dependent balance between liver damage and regeneration and the results showed that CR2-CD59 not only has no effect on the production of C3a and C5a but enhances liver regeneration and remarkably improves the long-term survival, partly because of the increased level of hepatic TNF-α and IL-6 via STAT3 and Akt activation [6].

IL-6 is a pleiotropic cytokine and is secreted during inflammatory conditions upon LPS stimulation in a TNF-α-dependent/-independent manner (Figure 2). In response to liver injury, IL-6 mediates the acute-phase response and induces both cytoprotective and mitogenic functions. IL-6-induced signaling pathways are critical to the early onset as well as the progression and maintenance of the regenerative process [9]. Conventionally, IL-6 binds to the interleukin-6 receptor (IL-6R) and the IL-6/IL-6R complex initiates a coreceptor, glycoprotein (gp) 130, leading to JAk/STAT, MAPK, and PI3K/AKT activation [10]. STAT3 is able to upregulate the expression of suppressors of cytokine signaling (SOCS), an important negative regulator of cytokine signaling, leading to the downregulation of gp130 signals [10, 11]. Surprisingly, either IL-6 or IL-6R alone has no affinity to gp130 and only when the IL-6/IL-6R complex is formed, interaction with gp130 would occur.

Although gp130 is present in almost all cells, IL-6R is only expressed in limited cell types, for example, hepatocyte; thus, it seems that the effect of IL-6 is restricted to these cells. However, a soluble form of IL-6R (sIL-6R) was found and could still bind IL-6 to trigger intracellular signals, being called IL-6 trans-signaling [12]. SIL-6R is mostly generated by the proteolytic cleavage of membrane-bound receptor or by alternative splicing of the transmembrane domain coding exon [13]. The event of IL-6 trans-signaling not only could occur on cells short of IL-6R but also affects hepatocytes expressing IL-6R to prolong STAT3 phosphorylation and enhances the effect of IL-6 in liver regeneration [14, 15]. Blockade of IL-6 trans-signaling would deteriorate CCL4-induced liver damage [16]. It has been speculated that sIL-6R and sgp130, a soluble form of gp130, may constitute a buffer in the blood and once secreted, IL-6 will bind sIL-6R and then the complex IL-6/sIL-6R will bind sgp130 with a high affinity. Only when the concentration of IL-6 is very high, exceeding the level of sIL-6R, IL-6 could bind to membrane-bound IL-6R [17].

Of note, researches recently found that gp130, independent of the gp130 effector STAT3, initiates the activation of YAP and Notch, controlling the tissue growth and regeneration in intestinal epithelial cells upon mucosal injury [18]. Besides, YAP overexpression has been found in several solid tumors and elevated YAP levels contribute to tumor growth. YAP-mediated induction of Jag-1 was able to activate Notch signaling in HCC and mouse hepatocytes [19]. The role of this novel signaling in liver injury, repair, and regeneration remains to be charted.

3. The Proliferation Phase: Complete Mitogens and Auxiliary Mitogens

The proliferation phase, also called the second phase or progression phase, converts cells from G1 phase to mitosis. The molecules involved in the second phase were mainly separated into two groups, that is, complete mitogens and auxiliary mitogens [20] (Table 1). The former refers to these factors mitogenic in both primary-cultured hepatocytes and in animal experiments, including hepatocyte growth factor (HGF), transforming growth factor- (TGF-) α, epidermal growth factor (EGF), heparin-binding-EGF (HB-EGF), and their common receptor EGFR. They could activate secondary or delayed gene responses to stimulate DNA synthesis and cell proliferation. The latter, although they are not mitogenic in hepatocytes, may contribute to the regenerative

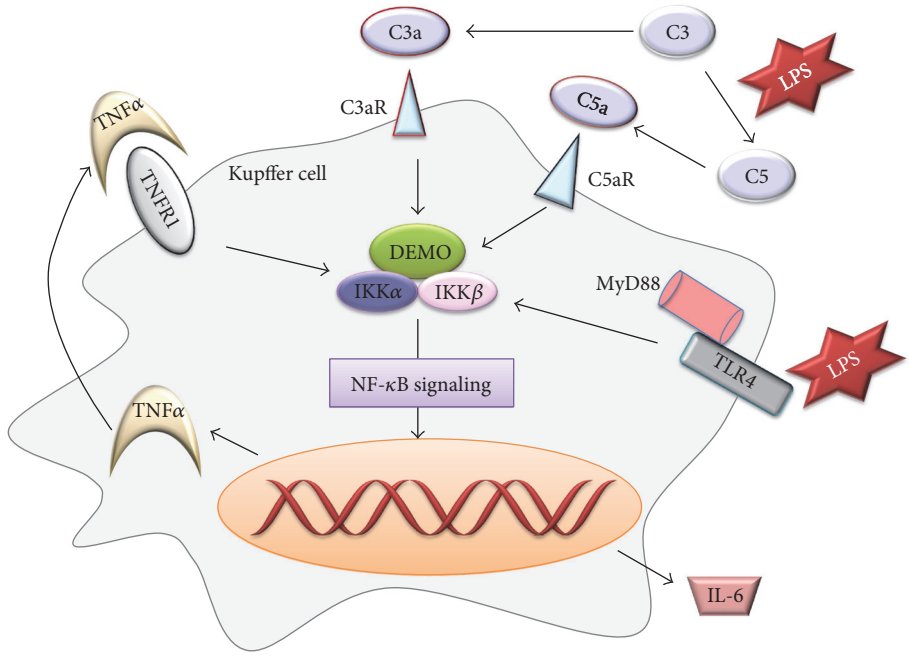

Figure 2: The production of TNF-α and IL-6 in Kupffer cell through NF-κB signaling in the early phase of liver regeneration.

process partially by magnifying or accelerating the effects of complete mitogens.

3.1. Complete Mitogens. Complete mitogens exhibit direct hepatotrophic effects, which is defined that they could lead DNA synthesis in serum-free media in vitro and cause liver enlargement when injected in vivo. HGF and ligands of EGFR, including EGF, TGF-α, and HB-EGF, acting as the major complete mitogens for hepatocytes, could provoke hepatocyte proliferation mainly through the Ras-MAPK signaling and PI3K/AKT signaling pathway by binding to corresponding receptors, c-met and EGFR [21, 22] (Figure 3). Based on the research conducted by Huh et al. [23], mice knockout of the c-met gene showed hypersensitivity to Fas-mediated apoptosis and may retard the development of the liver after injury. The similar condition was also observed when EGFR was suppressed by silencing RNAs [24].

3.2. Auxiliary Mitogens. Auxiliary mitogens, such as bile acids (BAs) [25], norepinephrine (NE) [26], endothelial growth factor (VEGF) [27], insulin-like growth factor system (IGF system) [28], estrogen [29], and serotonin [30] (Table 1), although not mitogenic in cultured hepatocytes, may delay liver regeneration in their absence.

Blood platelets, not just functioning in the hematologic system, actually fulfill a wider role in health and diseases [42]. Platelets may be part of the innate immune system and also fight with infection, including bacteria, viruses, and microorganisms. Mediators provided by platelets not only recruit leukocytes to the site of vascular injury and inflammation but also aid in tissue repair and regeneration. Being recruited to the sinusoids after PHx and releasing molecules, such as HGF, VEGF, insulin-like growth factor-1 (IGF-1), and serotonin, platelets are described as a positive factor involved in liver regeneration [43]. Patients suffering from 70% PHx would improve the regenerative capacity of the liver if provided with plasma rich in platelets [44]. Conversely, administration of antiplatelet antibodies would depress liver regeneration [45]. However, it does not mean that administration of platelet concentrates and thrombopoietin receptor agonists could be widely used on clinical operations to support liver regeneration and alleviate outcomes of patients with liver failure or small-for-size syndrome owing to the severely undesirable side effects brought by the strategy, for example, venous or portal vein thrombosis, or even fatal transfusion-related acute lung injury [46]. Thus, more works are needed to ascertain its beneficial effects and to minimize potential side effects at the same time.

3.3. Wnt Proteins. Wnt ligands are secreted glycoproteins and are produced primarily by hepatic nonparenchymal cell compartment, especially Kupffer cells and endothelial cells [47]. They are beneficial and necessary for liver regeneration. Wnts may activate the chief downstream effector, β-catenin, and initiate the classic wnt/β-catenin signaling cascade and finally express target genes, such as *c-myc* and cyclinD1 [48]. Other than wnt proteins, β-catenin can also be stimulated through a non-wnt fashion, that is, wnt-independent signaling. β-Catenin forms the bridge between the cytoplasmic tail of E-cadherin and actin cytoskeleton, through which β-catenin may act as a mediator of tyrosine kinase signaling [49]. At the membrane, β-catenin could be phosphorylated at tyrosine residues 654 and 670 by different kinases including c-met, EGFR, and others [50], which induce the dissociation of β-catenin from E-cadherin, and subsequently, β-catenin translocates to the nucleus to control the expression of target genes (Figure 3). Although both classic wnt/β-catenin signaling and wnt-independent signaling are

Table 1: Common complete and auxiliary mitogens.

Factor	Origin	Target
Complete mitogens		
HGF	Mainly stellate cells	HGF directly regulates hepatocyte DNA synthesis and cell proliferation by blinding to its receptor c-met.
EGF	Brunner's gland in the duodenum	They provoke hepatocyte proliferation mainly through the Ras-MAPK signaling pathway by binding to their identical receptor and may compensate for each other to some degree in the process.
TGF-α	Hepatocytes	
Auxiliary mitogens		
Bile acids	Hepatocytes and cholangicytes	Appropriate concentration of BAs may promote liver regeneration mainly via farnesoid X receptor (FXR) signaling pathways to stimulate the expression of FoxM1b, a key regulator of cell cycle, to participate in cells proliferation [31].
NE	Nerve system	NE may amplify the effect of EGF and HGF by acting on the α1-adrenergic receptor associated with $G\alpha_h$, a G protein [32, 33], and besides, it could induced the expression of Smad7 to abolish activin A-induced growth inhibition of hepatocyte by activation of NF-κB [34].
VEGF	Hepatocytes	VEGF family, particularly VEGF-A, is strongly upregulated in hepatocytes during the regenerative process and may facilitate proliferation of sinusoidal endothelial cells and hepatocytes 48 h following PHx [35].
Insulin	Pancreatic islets	Insulin could contribute to liver regeneration despite not being a primary mitogen and its proliferative effect mainly mediated through insulin receptors (IRs) that shift to nucleus to activate inositol 1,4,5,-trisphosphate-(InsP3-) dependent Ca^{2+} signaling pathways [36].
IGF-1	Liver	IGF-I works as a booster to liver regeneration by upregulation of HGF and downregulation of transforming growth factor beta 1(TGF-β1), a repressor of proliferation, and decreased level of IGF-I could impair the regenerative process [37].
Estrogen	Reproductive system	Estrogen has been shown to promote hepatocyte proliferation mainly through estrogen receptor alpha (ERα) [38]. Moreover, the estrogen level could be influenced by IL-6 and there may be crosstalk between estrogen signaling and IL-6 signaling pathways [39].
Serotonin (5-hydroxytryptamine, 5HT)	Enterochromaffin cells	Serotonin, via HT receptor 2 (HTR2), has been reported to contribute to liver regeneration [40]. And it was found that liver regeneration would be arrested when ketanserin was administrated to block 5-HT2, a subtype of 5-HT, approximately at the G1/S transition point [41].

advantageous to the regenerative process, the positive role of the former is more remarkable. When knocked out of LRP5/6, coreceptor of wnt proteins, mice showed impaired classic wnt/β-catenin signaling and retarded regenerative process after PHx despite that the non-wnt pathways remained intact [51].

3.4. Exosomes. Other than hormones, cytokines, and growth factors contributing to liver regeneration, exosomes are found to improve the regenerative process as well. Exosomes are membrane-enclosed nanovesicles possessing a variety of physiological properties and function as important vesicles involved in intercellular communication [52]. Exosomes are released by several types of cells and carry active signals to target cell within adjacent and remote areas. Recently, exosomes derived from hepatocytes were reported to improve liver regeneration owing to the production of intracellular sphingosine-1-phosphate (S1P) [53]. S1P is indispensable for hepatocyte exosome-induced proliferation. Only hepatocyte-derived exosomes, not other liver cells, contain neutral ceramidase and sphingosine kinase 2 (SK2) required for S1P synthesis. Exosomes fuse with and deliver synthetic machinery to target hepatocyte. And within the target hepatocyte, sphingosine-1-phosphate (S1P) is produced to promote cell proliferation. Besides, the number of hepatocyte-derived exosomes increased after liver injury. Similarly, MSC-derived exosomes exert hepatoprotective effects and relieve drug-induced liver injury through activation of proliferative and regenerative responses [54], underlining the tremendous potential of the exosome-based therapies for liver disease.

4. Termination of Liver Regeneration

When the normal liver mass/body mass ratio of 2.5% has been restored, liver regeneration would be terminated. However, mechanisms of controlling the hepatocyte apoptosis to correct an overshooting of regenerative response have not been well investigated. Thus far, the most well-known

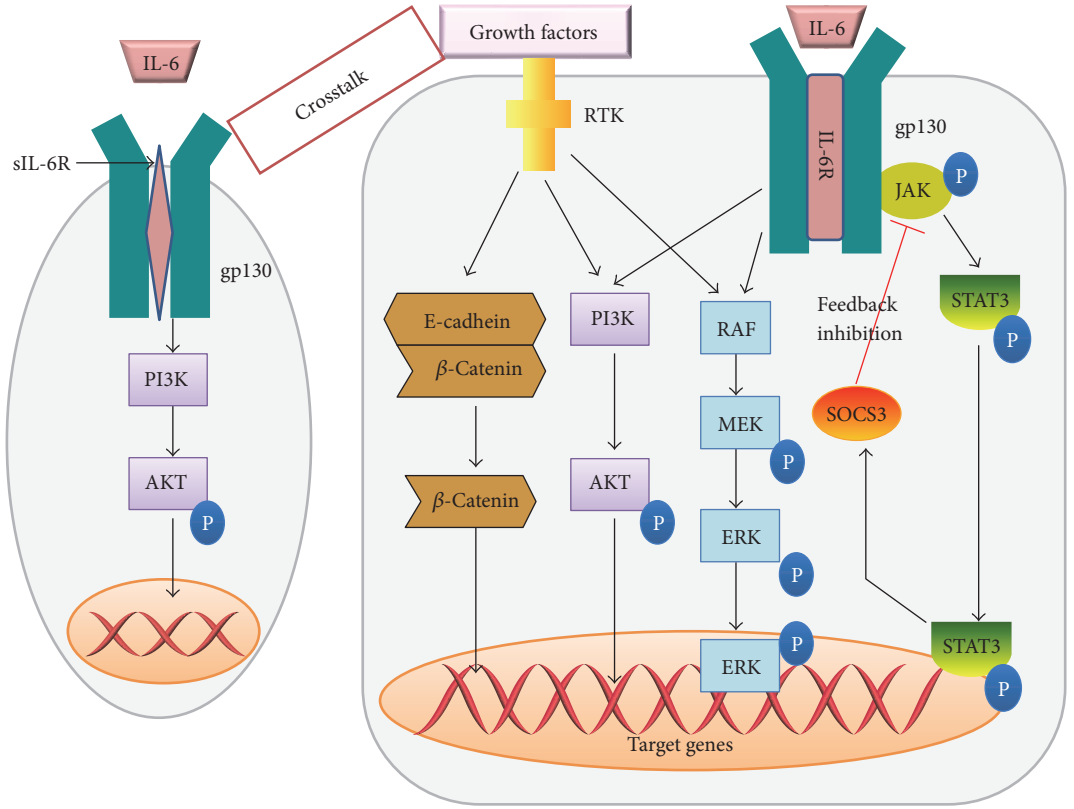

Figure 3: Growth factors, along with some cytokines, guide the progression of liver regeneration through expression of some cell cycle-related proteins mainly by PI3K/AKT, wnt-independent/β-catenin, Ras/MAPK, and JAK/STAT signaling pathways.

antiproliferative factors are transforming growth factor beta (TGF-β) and related TGF-β family members [55].

4.1. TGF-β-Mediated Pathways. TGF-β, especially TGF-β1, puts a brake on liver regeneration and works as an inducer of cell apoptosis in vitro and in vivo, being active in G1 phase of the cell cycle [56]. TGF-β1 exerts its function mainly through binding to its receptors type I receptor (TβRI) and type II receptor (TβRII) to encode related protein expression. Whereas, researchers recently have proved the lack of TGF-β gene upregulation in the termination stage and concluded that intact signaling by TGF-β may not be required for the termination phase of liver regeneration [57]. They also found that some genes were upregulated in the termination regulation and may have potential negative effect on the cell cycle and promotion of cell apoptosis, such as the zinc finger protein gene (ZNF490) and caspase recruitment domain-containing protein 11 (CARD11) gene. Therefore, more details are needed to verify and elucidate molecules that participated in the termination phase and the signaling they involved.

Apart from liver cells, TGF-β1 is also synthesized in extrahepatic tissues, including platelet and the spleen [58, 59]. The spleen, known as an immune organ, would secret TGF-β1 to end the liver regeneration. Splenectomy significantly increased the number of proliferating cells 48 h after PHx [60]. Recently, the spleen was proved to not only increase TGF-β1and its receptor TβRII but also downregulate HGF and its receptor c-met to exhibit growth inhibitory effects on cell proliferation, indicating that the spleen could remotely influence and regulate liver regeneration [59].

4.2. Other Relevant TGF-β Family Members. Other TGF-β family members, primarily activins and bone morphogenetic proteins (BMPs), were revealed to be implicated in numerous biological processes, including liver regeneration. Activin A, an activin subtype, is increased by 12 h in response to partial hepatectomy and considered as a negative regulator of liver regeneration and induces hepatocyte growth arrest and apoptosis in vitro and in vivo [61, 62]. Administration of the activin A antagonist follistatin enhanced DNA synthesis and prolonged hepatocyte proliferation [63]. Apart from the inhibitory effect on DNA synthesis, activin A also significantly affects the production of fibronectin, component of extracellular matrix (ECM) which is essential for liver regeneration [64]. With regard to BMP, unlike TGF-β1 and activin A, it is quite complicated. Major BMPs bind to their receptors mainly to phosphorylate Smad1/5/8 rather than Smad2/3 to exert repressive effects on liver regeneration [65]. However, different subunits of BMPs exhibit different or even reverse effects, for example, BMP7 that promotes hepatocyte proliferation, whereas BMP4 represses proliferation in the hepatoma cell line Huh7 [66]. One main possible reason may be that BMP7 and BMP4 act through pathways of opposite effects [67]. Thus, the accurate functions of other BMPs in the liver regeneration process remain to be undetermined.

5. The Future Perspectives in the Fields of Liver Regeneration

In spite of having been investigated for so many years, the actual mechanisms of liver regeneration are still obscure and far from practical application to solve clinical liver disease. However, the embarrassing situation is greatly improved by the appearance of new-fashioned technologies—cell transplantation therapy and liver bioengineering—aimed at alleviating the dilemma caused by insufficient liver regeneration or shortage of liver donors, and they are becoming hotspots in the research field. Cell transplantation, mainly referring to stem cells or progenitor cells, has been extensively studied on liver regeneration owing to the potential of differentiation into hepatocytes [68]. Studies have demonstrated that mesenchymal stem cells (MSC) [69], fetal progenitor cell [70], and embryonic stem cells [71] could improve liver injury to some extent. However, the source of transplanted cells and the livability and immune rejection after being transplanted may limit the application of cell therapy on clinical operations. Whereas, liver bioengineering, namely, three-dimensional matrix liver scaffolds, was first reported in 2010 and it includes two parts: decellularization and recellularization [72]. A decellularized liver scaffold (DLS) is characterized by retaining intact vasculature system and a fine web of matrix, providing necessary environment similar to a normal liver for cells to grow, proliferate, and differentiate [73]. After that, the DLS would be repopulated with functional human cells, mainly autologous liver progenitor cells. Furthermore, perfusion of the recellularized liver scaffold with positive molecules for cell regeneration or differentiation, for example, granulocyte colony stimulating factor (G-CSF), may facilitate liver regeneration [74]. Taken together, cell therapy, along with liver bioengineering, may be a new path for liver regeneration development.

6. Conclusions

Despite having been studied for so many years, the passion and energy for liver regeneration never fade out, since the demands for it is urgent from the past till now because of liver transplantation or liver failure or other end-stage liver diseases. It is a multifactor and multipath network, and the exact mechanisms are incompletely understood. Although the appearance of the new technologies opens our thoughts and horizons and, together with the previous results of researches, may drive us closer to clinical application, we still have a long way to go as we always operated studies on animals and the conclusions we deduced could not be applied to human directly because of species differences.

Abbreviations

TGF-β: Transforming growth factor beta
TNF-α: Tumor necrosis factor-α
TLR: Toll-like receptor
MyD88: Myeloid differentiation factor 88
AP-1: Activating protein1
LPS: Lipopolysaccharide
SOCS: Suppressors of cytokine signaling
HGF: Hepatocyte growth factor
TGF: Transforming growth factor
EGF: Epidermal growth factor
BAs: Bile acids
NE: Norepinephrine
VEGF: Endothelial growth factor
IGF: Insulin-like growth factor
ER: Estrogen receptors
ZNF490: Zinc finger protein gene
CARD11: Caspase recruitment domain-containing protein 11
ECM: Extracellular matrix
S1P: Sphingosine-1-phosphate
SK2: Sphingosine kinase 2
MSC: Mesenchymal stem cells
DLS: Decellularized liver scaffold
G-CSF: Granulocyte-colony stimulating factor.

Conflicts of Interest

The authors have no financial conflict of interests.

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References

[1] G. K. Michalopoulos and M. C. DeFrances, "Liver regeneration," *Science*, vol. 276, no. 5309, pp. 60–66, 1997.

[2] G. P. Sun and K. D. Irvine, "Control of growth during regeneration," *Current Topics in Developmental Biology*, vol. 108, pp. 95–120, 2014.

[3] P. S. Pahlavan, R. E. Feldmann, C. Zavos, and J. Kountouras, "Prometheus' challenge: molecular, cellular and systemic aspects of liver regeneration," *Journal of Surgical Research*, vol. 134, no. 2, pp. 238–251, 2006.

[4] M. Karin and H. Clevers, "Reparative inflammation takes charge of tissue regeneration," *Nature*, vol. 529, no. 7586, pp. 307–315, 2016.

[5] L. I. Kang, W. M. Mars, and G. K. Michalopoulos, "Signals and cells involved in regulating liver regeneration," *Cell*, vol. 1, no. 4, pp. 1261–1292, 2012.

[6] K. M. Marshall, S. He, Z. Zhong, C. Atkinson, and S. Tomlinson, "Dissecting the complement pathway in hepatic injury and regeneration with a novel protective strategy," *The Journal of Experimental Medicine*, vol. 211, no. 9, pp. 1793–1805, 2014.

[7] D. Mastellos, J. C. Papadimitriou, S. Franchini, P. A. Tsonis, and J. D. Lambris, "A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration," *Journal of Immunology*, vol. 166, no. 4, pp. 2479–2486, 2001.

[8] C. W. Strey, M. Markiewski, D. Mastellos et al., "The proinflammatory mediators C3a and C5a are essential for liver regeneration," *The Journal of Experimental Medicine*, vol. 198, no. 6, pp. 913–923, 2003.

[9] M. Fujiyoshi and M. Ozaki, "Molecular mechanisms of liver regeneration and protection for treatment of liver dysfunction and diseases," *Journal of Hepato-Biliary-Pancreatic Surgery*, vol. 18, no. 1, pp. 13–22, 2011.

[10] F. Schaper and S. Rose-John, "Interleukin-6: biology, signaling and strategies of blockade," *Cytokine & Growth Factor Reviews*, vol. 26, no. 5, pp. 475–487, 2015.

[11] J. S. Campbell, L. Prichard, F. Schaper et al., "Expression of suppressors of cytokine signaling during liver regeneration," *The Journal of Clinical Investigation*, vol. 107, no. 10, pp. 1285–1292, 2001.

[12] Y. Nechemia-Arbely, A. Shriki, U. Denz et al., "Early hepatocyte DNA synthetic response posthepatectomy is modulated by IL-6 trans-signaling and PI3K/AKT activation," *Journal of Hepatology*, vol. 54, no. 5, pp. 922–929, 2011.

[13] J. Wolf, S. Rose-John, and C. Garbers, "Interleukin-6 and its receptors: a highly regulated and dynamic system," *Cytokine*, vol. 70, no. 1, pp. 11–20, 2014.

[14] M. Peters, G. Blinn, T. Jostock et al., "Combined interleukin 6 and soluble interleukin 6 receptor accelerates murine liver regeneration," *Gastroenterology*, vol. 119, no. 6, pp. 1663–1671, 2000.

[15] C. Drucker, J. Gewiese, S. Malchow, J. Scheller, and S. Rose-John, "Impact of interleukin-6 classic- and trans-signaling on liver damage and regeneration," *Journal of Autoimmunity*, vol. 34, no. 1, pp. 29–37, 2010.

[16] J. Gewiese-Rabsch, C. Drucker, S. Malchow, J. Scheller, and S. Rose-John, "Role of IL-6 trans-signaling in CCl_4 induced liver damage," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, vol. 1802, no. 11, pp. 1054–1061, 2010.

[17] D. Schmidt-Arras and S. Rose-John, "IL-6 pathway in the liver: from physiopathology to therapy," *Journal of Hepatology*, vol. 64, no. 6, pp. 1403–1415, 2016.

[18] K. Taniguchi, L. W. Wu, S. I. Grivennikov et al., "A gp130-Src-YAP module links inflammation to epithelial regeneration," *Nature*, vol. 519, no. 7541, pp. 57–62, 2015.

[19] D. F. Tschaharganeh, X. Chen, P. Latzko et al., "Yes-associated protein up-regulates Jagged-1 and activates the Notch pathway in human hepatocellular carcinoma," *Gastroenterology*, vol. 144, no. 7, pp. 1530–1542.e12, 2013.

[20] G. K. Michalopoulos, "Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas," *The American Journal of Pathology*, vol. 176, no. 1, pp. 2–13, 2010.

[21] S. A. Mao, J. M. Glorioso, and S. L. Nyberg, "Liver regeneration," *Translational Research*, vol. 163, no. 4, pp. 352–362, 2014.

[22] F. Hong, V. A. Nguyen, X. Shen, G. Kunos, and B. Gao, "Rapid activation of protein kinase B/Akt has a key role in antiapoptotic signaling during liver regeneration," *Biochemical and Biophysical Research Communications*, vol. 279, no. 3, pp. 974–979, 2000.

[23] C. G. Huh, V. M. Factor, A. Sanchez, K. Uchida, E. A. Conner, and S. S. Thorgeirsson, "Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 13, pp. 4477–4482, 2004.

[24] S. Paranjpe, W. C. Bowen, G. C. Tseng, J. H. Luo, A. Orr, and G. K. Michalopoulos, "RNA interference against hepatic epidermal growth factor receptor has suppressive effects on liver regeneration in rats," *American Journal of Pathology*, vol. 176, no. 6, pp. 2669–2681, 2010.

[25] W. D. Huang, K. Ma, J. Zhang et al., "Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration," *Science*, vol. 312, no. 5771, pp. 233–236, 2006.

[26] J. L. Cruise, "Alpha-1-adrenergic receptors in liver-regeneration," *Digestive Diseases and Sciences*, vol. 36, no. 4, pp. 485–488, 1991.

[27] M. Bockhorn, M. Goralski, D. Prokofiev et al., "VEGF is important for early liver regeneration after partial hepatectomy," *The Journal of Surgical Research*, vol. 138, no. 2, pp. 291–299, 2007.

[28] M. Enguita-German and P. Fortes, "Targeting the insulin-like growth factor pathway in hepatocellular carcinoma," *World Journal of Hepatology*, vol. 6, no. 10, pp. 716–737, 2014.

[29] L. Biondo-Simoes Mde, T. R. Erdmann, S. O. Ioshii, J. E. Matias, H. L. Calixto, and D. J. Schebelski, "The influence of estrogen on liver regeneration: an experimental study in rats," *Acta Cirurgica Brasileira / Sociedade Brasileira para Desenvolvimento Pesquisa em Cirurgia*, vol. 24, no. 1, pp. 3–6, 2009.

[30] P. A. Clavien, "Liver regeneration: a spotlight on the novel role of platelets and serotonin," *Swiss Medical Weekly*, vol. 138, no. 25-26, pp. 361–370, 2008.

[31] M. J. Fan, X. C. Wang, G. Y. Xu, Q. Yan, and W. Huang, "Bile acid signaling and liver regeneration," *Biochimica et Biophysica Acta-Gene Regulatory Mechanisms*, vol. 1849, no. 2, pp. 196–200, 2015.

[32] P. M. Lindroos, R. Zarnegar, and G. K. Michalopoulos, "Hepatocyte growth-factor (hepatopoietin A) rapidly increases in plasma before DNA-synthesis and liver-regeneration stimulated by partial-hepatectomy and carbon-tetrachloride administration," *Hepatology*, vol. 13, no. 4, pp. 743–750, 1991.

[33] Y. Ohtake, T. Kobayashi, A. Maruko et al., "Norepinephrine modulates the zonally different hepatocyte proliferation through the regulation of transglutaminase activity," *American Journal of Physiology Gastrointestinal and Liver Physiology*, vol. 299, no. 1, pp. G106–G114, 2010.

[34] C. Kanamaru, H. Yasuda, M. Takeda et al., "Smad7 is induced by norepinephrine and protects rat hepatocytes from activin A-induced growth inhibition," *The Journal of Biological Chemistry*, vol. 276, no. 49, pp. 45636–45641, 2001.

[35] E. Taniguchi, S. Sakisaka, K. Matsuo, K. Tanikawa, and M. Sata, "Expression and role of vascular endothelial growth factor in liver regeneration after partial hepatectomy in rats," *The Journal of Histochemistry and Cytochemistry : Official Journal of the Histochemistry Society*, vol. 49, no. 1, pp. 121–130, 2001.

[36] M. J. Amaya, A. G. Oliveira, E. S. Guimaraes et al., "The insulin receptor translocates to the nucleus to regulate cell proliferation in liver," *Hepatology*, vol. 59, no. 1, pp. 274–283, 2014.

[37] G. Wallek, N. Friedrich, T. Ittermann et al., "IGF-1 and IGFBP-3 in patients with liver disease," *Laboratoriumsmedizin*, vol. 37, no. 1, pp. 13–20, 2013.

[38] T. Uebi, M. Umeda, and T. Imai, "Estrogen induces estrogen receptor alpha expression and hepatocyte proliferation in the livers of male mice," *Genes to Cells : Devoted to Molecular & Cellular Mechanisms*, vol. 20, no. 3, pp. 217–223, 2015.

[39] E. J. Chiu, H. L. Lin, C. W. Chi, T. Y. Liu, and W. Y. Lui, "Estrogen therapy for hepatectomy patients with poor liver function?," *Medical Hypotheses*, vol. 58, no. 6, pp. 516–518, 2002.

[40] M. Lesurtel and P. A. Clavien, "Serotonin: a key molecule in acute and chronic liver injury!," *Clinics and Research in Hepatology and Gastroenterology*, vol. 36, no. 4, pp. 319–322, 2012.

[41] G. K. Papadimas, K. N. Tzirogiannis, M. G. Mykoniatis, A. D. Grypioti, G. A. Manta, and G. I. Panoutsopoulos, "The emerging role of serotonin in liver regeneration," *Swiss Medical Weekly*, vol. 142, article w13548, 2012.

[42] A. T. Nurden, "Platelets, inflammation and tissue regeneration," *Thrombosis and Haemostasis*, vol. 105, Supplement 1, pp. S13–S33, 2011.

[43] J. Meyer, E. Lejmi, P. Fontana, P. Morel, C. Gonelle-Gispert, and L. Bühler, "A focus on the role of platelets in liver regeneration: do platelet-endothelial cell interactions initiate the regenerative process?," *Journal of Hepatology*, vol. 63, no. 5, pp. 1263–1271, 2015.

[44] R. Matsuo, Y. Nakano, and N. Ohkohchi, "Platelet administration via the portal vein promotes liver regeneration in rats after 70% hepatectomy," *Annals of Surgery*, vol. 253, no. 4, pp. 759–763, 2011.

[45] S. Murata, N. Ohkohchi, R. Matsuo, O. Ikeda, A. Myronovych, and R. Hoshi, "Platelets promote liver regeneration in early period after hepatectomy in mice," *World Journal of Surgery*, vol. 31, no. 4, pp. 808–816, 2007.

[46] T. Lisman and R. J. Porte, "Mechanisms of platelet-mediated liver regeneration," *Blood*, vol. 128, no. 5, pp. 625–629, 2016.

[47] B. S. Ding, D. J. Nolan, J. M. Butler et al., "Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration," *Nature*, vol. 468, no. 7321, pp. 310–U240, 2010.

[48] S. P. S. Monga, "Role of Wnt/β-catenin signaling in liver metabolism and cancer," *The International Journal of Biochemistry & Cell Biology*, vol. 43, no. 7, pp. 1021–1029, 2011.

[49] S. S. Monga, "Role and regulation of β-catenin signaling during physiological liver growth," *Gene Expression*, vol. 16, no. 2, pp. 51–62, 2014.

[50] K. N. Nejak-Bowen and S. P. S. Monga, "Beta-catenin signaling, liver regeneration and hepatocellular cancer: sorting the good from the bad," *Seminars in Cancer Biology*, vol. 21, no. 1, pp. 44–58, 2011.

[51] J. Yang, L. E. Mowry, K. N. Nejak-Bowen et al., "Beta-catenin signaling in murine liver zonation and regeneration: a Wnt-Wnt situation!," *Hepatology*, vol. 60, no. 3, pp. 964–976, 2014.

[52] G. Lou, Z. Chen, M. Zheng, and Y. Liu, "Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases," *Experimental & Molecular Medicine*, vol. 49, no. 6, article e346, 2017.

[53] H. Nojima, C. M. Freeman, R. M. Schuster et al., "Hepatocyte exosomes mediate liver repair and regeneration via sphingosine-1-phosphate," *Journal of Hepatology*, vol. 64, no. 1, pp. 60–68, 2016.

[54] C. Y. Tan, R. C. Lai, W. Wong, Y. Y. Dan, S. K. Lim, and H. K. Ho, "Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models," *Stem Cell Research & Therapy*, vol. 5, no. 3, p. 76, 2014.

[55] R. Derynck and Y. E. Zhang, "Smad-dependent and Smad-independent pathways in TGF-β family signalling," *Nature*, vol. 425, no. 6958, pp. 577–584, 2003.

[56] J. Romero-Gallo, E. G. Sozmen, A. Chytil et al., "Inactivation of TGF-β signaling in hepatocytes results in an increased proliferative response after partial hepatectomy," *Oncogene*, vol. 24, no. 18, pp. 3028–3041, 2005.

[57] I. E. Nygard, K. E. Mortensen, J. Hedegaard et al., "The genetic regulation of the terminating phase of liver regeneration," *Comparative Hepatology*, vol. 11, no. 1, p. 3, 2012.

[58] K. Takahashi, S. Murata, and N. Ohkohchi, "Novel therapy for liver regeneration by increasing the number of platelets," *Surgery Today*, vol. 43, no. 10, pp. 1081–1087, 2013.

[59] S. C. Lee, H. J. Jeong, B. J. Choi, and S. J. Kim, "Role of the spleen in liver regeneration in relation to transforming growth factor-β1 and hepatocyte growth factor," *Journal of Surgical Research*, vol. 196, no. 2, pp. 270–277, 2015.

[60] S. Ueda, A. Yamanoi, Y. Hishikawa, D. K. Dhar, M. Tachibana, and N. Nagasue, "Transforming growth factor-β1 released from the spleen exerts a growth inhibitory effect on liver regeneration in rats," *Laboratory Investigation*, vol. 83, no. 11, pp. 1595–1603, 2003.

[61] K. Takamura, K. Tsuchida, H. Miyake, S. Tashiro, and H. Sugino, "Activin and activin receptor expression changes in liver regeneration in rat," *Journal of Surgical Research*, vol. 126, no. 1, pp. 3–11, 2005.

[62] L. Chen, W. Zhang, H. F. Liang et al., "Activin A induces growth arrest through a SMAD-dependent pathway in hepatic progenitor cells," *Cell Communication and Signaling: CCS*, vol. 12, p. 18, 2014.

[63] K. Kogure, W. Omata, M. Kanzaki et al., "A single intraportal administration of follistatin accelerates liver-regeneration in partially hepatectomized rats," *Gastroenterology*, vol. 108, no. 4, pp. 1136–1142, 1995.

[64] M. Date, K. Matsuzaki, M. Matsushita, Y. Tahashi, K. Sakitani, and K. Inoue, "Differential regulation of activin A for hepatocyte growth and fibronectin synthesis in rat liver injury," *Journal of Hepatology*, vol. 32, no. 2, pp. 251–260, 2000.

[65] D. Tsugawa, Y. Oya, R. Masuzaki et al., "Specific activin receptor-like kinase 3 inhibitors enhance liver regeneration," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 351, no. 3, pp. 549–558, 2014.

[66] N. G. Kan, D. Junghans, and J. C. I. Belmonte, "Compensatory growth mechanisms regulated by BMP and FGF signaling mediate liver regeneration in zebrafish after partial hepatectomy," *The FASEB Journal*, vol. 23, no. 10, pp. 3516–3525, 2009.

[67] N. Do, R. Zhao, K. Ray et al., "BMP4 is a novel paracrine inhibitor of liver regeneration," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, vol. 303, no. 11, pp. G1220–G1227, 2012.

[68] Z. Zhang and F. S. Wang, "Stem cell therapies for liver failure and cirrhosis," *Journal of Hepatology*, vol. 59, no. 1, pp. 183–185, 2013.

[69] M. Esrefoglu, "Role of stem cells in repair of liver injury: experimental and clinical benefit of transferred stem cells on liver failure," *World Journal of Gastroenterology*, vol. 19, no. 40, pp. 6757–6773, 2013.

[70] T. Kisseleva, E. Gigante, and D. A. Brenner, "Recent advances in liver stem cell therapy," *Current Opinion in Gastroenterology*, vol. 26, no. 4, pp. 395–402, 2010.

[71] T. Ezzat, D. K. Dhar, M. Malago, and S. W. Olde Damink, "Dynamic tracking of stem cells in an acute liver failure

model," *World Journal of Gastroenterology*, vol. 18, no. 6, pp. 507–516, 2012.

[72] B. E. Uygun, A. Soto-Gutierrez, H. Yagi et al., "Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix," *Nature Medicine*, vol. 16, no. 7, pp. 814–U120, 2010.

[73] L. F. Tapias and H. C. Ott, "Decellularized scaffolds as a platform for bioengineered organs," *Current Opinion in Organ Transplantation*, vol. 19, no. 2, pp. 145–152, 2014.

[74] C. Booth, T. Soker, P. Baptista et al., "Liver bioengineering: current status and future perspectives," *World Journal of Gastroenterology*, vol. 18, no. 47, pp. 6926–6934, 2012.

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Research Article

Amelioration of Ethanol-Induced Hepatitis by Magnesium Isoglycyrrhizinate through Inhibition of Neutrophil Cell Infiltration and Oxidative Damage

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Alcoholic liver disease (ALD) is a leading cause of liver-related morbidity and mortality worldwide. There is no effective treatment to prevent the disease progression. Magnesium isoglycyrrhizinate (MgIG) showed potent anti-inflammatory, antioxidant, and hepatoprotective activities and was used for treating liver diseases in Asia. In this study, we examined whether MgIG could protect mice against alcohol-induced liver injury. The newly developed chronic plus binge ethanol feeding model was used to study the role of MgIG in ALD. Serum liver enzyme levels, H&E staining, immunohistochemical staining, flow cytometric analysis, and real-time PCR were used to evaluate the liver injury and inflammation. We showed that MgIG markedly ameliorated chronic plus binge ethanol feeding liver injury, as shown by decreased serum alanine transaminase and aspartate aminotransferase levels and reduced neutrophil infiltration. The reason may be attributed to the reduced expression of proinflammatory cytokines and chemokines with the treatment of MgIG. The hepatoprotective effect of MgIG was associated with suppression of neutrophil ROS production as well as hepatocellular oxidative stress. MgIG may play a critical role in protecting against chronic plus binge ethanol feeding-induced liver injury by regulating neutrophil activity and hepatic oxidative stress.

1. Introduction

The prevalence of chronic alcohol consumption has increased in the last decades in the Western world as well as in Asian countries [1]. According to the WHO report in 2011, chronic alcohol consumption resulted in approximately 2.5 million deaths each year. Among these alcoholics, almost 20% of them developed alcoholic liver disease (ALD), which was still a leading cause of liver-related morbidity and mortality worldwide. The pathogenesis of ALD was a complex process in both parenchymal and nonparenchymal cells and other cell types recruited into the liver in response to liver damage and inflammation. Hepatocytes were damaged by increased ethanol via generation of reactive oxygen species (ROS), endoplasmic reticulum (ER) stress, and mitochondrial dysfunction [2]. The damaged hepatocytes release danger-associated molecular patterns (DAMPs), together with pathogen-associated molecular patterns (PAMPs) derived from gut bacteria due to the increased permeability by ethanol, triggered liver inflammation, and recruited neutrophils into the liver [2, 3]. The accumulation of neutrophils in the liver promoted further hepatocellular injury and inflammation which was believed to be critical in the development of ALD [4, 5]. The conventional treatment of ALD such as corticosteroids or tumor necrosis factor alpha (TNF-α) inhibitor therapy usually causes increased chance of infections since these drugs were immune suppressive. So, it is very important to explore novel strategies for treating ALD [3].

Magnesium isoglycyrrhizinate (MgIG), a magnesium salt of 18α-glycyrrhizic acid stereoisomer of glycyrrhizic acid, is clinically used for the treatment of inflammatory liver

diseases in China and Japan [6–8]. MgIG has been reported to have strong anti-inflammatory, antioxidant, antiviral, and hepatoprotective activities [9–11]. MgIG may inhibit LPS-induced activation of phospholipase A_2 (PLA_2)/arachidonic acid (AA) pathway. Treatment of MgIG suppressed the production of AA metabolites induced by LPS, such as prostaglandin E2 (PGE2), prostacyclin (PGI2), thromboxane 2 (TXB2), and leukotrienes (LTB4) in macrophages [11]. Other studies indicated that MgIG inhibits inflammatory response through blocking STAT3 pathway activation in partial hepatectomy model and ischemia/reperfusion liver injury model [9, 12]. MgIG also showed hepatoprotective effects in drug-induced liver injury [13, 14], immune-mediated liver injury [10], and fatty liver [15]. A recent report showed that MgIG could reduce lipid accumulation induced by ethanol in vitro [16]; however, whether MgIG can be used for effectively treating ALD in vivo remains unknown.

To mimic acute-on-chronic alcoholic liver injury in patients, Bertola et al. described a novel mouse chronic plus binge ethanol feeding model (NIAAA model) for ALD [17]. Briefly, mice were subjected by chronic ethanol feeding (10 d ad libitum oral feeding with the Lieber-DeCarli ethanol liquid diet) plus a single binge dose of ethanol delivered by gavage. This model reproduced the drinking behaviors of ALD patients with elevated serum levels of alanine aminotransferase (ALT), steatosis, and neutrophil infiltration in the liver and upregulated the expression of proinflammatory cytokines. In this study, we utilized this NIAAA model to investigate the protective effects and mechanism underlying the effect of MgIG on ALD.

2. Materials and Methods

2.1. Materials. MgIG powder was provided by Nanjing Zhengda Tianqing Pharmaceutical Co. Ltd., Nanjing, China. MgIG powder was dissolved in PBS for injections.

2.2. Animals and NIAAA Model. Adult male C57BL/6 mice weighing 20–25 g used in this study were used for ad libitum ethanol feeding, described as the chronic plus binge alcohol feeding [17]. Lieber-DeCarli '82 Shake and Pour control liquid diet and Lieber-DeCarli '82 Shake and Pour ethanol liquid diet (Bio-Serv, Frenchtown, NJ) were prepared according to the manufacturer's instruction. Mice were fed with liquid control diet for 5 days and then switched either to a liquid diet containing 5% ethanol or a control diet for 10 days. MgIG (22.5 mg/kg or 45 mg/kg) or PBS was administered i.p. every day during the 10-day liquid diet or 10-day control diet. At day 11, mice were treated with MgIG or PBS; 2 hours later, all mice were gavaged with a single dose of ethanol (5 g/kg) or isocaloric maltodextrin. All mice were sacrificed 9 hours after gavage. The experiment was carried out with the approval of the institution animal use committee.

2.3. Histopathologic Evaluation. Liver specimens were collected and fixed in 10% formalin and paraffin embedded, then cut into 4 μm slices, and stained with hematoxylin and eosin (H&E) and immunohistochemistry for MPO, HNE, and MDA using a rabbit ABC staining kit (Vector Laboratories, Inc., Burlingame, CA) according to the manufacturer's protocol. Primary antibodies used were listed below: antimyeloperoxidase (MPO) (Biocare Medical, Concord, CA), antimalonaldehyde (MDA) (Genox, Baltimore, MD), and 4-hydroxynonenal (4-HNE) (Genox).

2.4. Biochemical Assays. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were analysed using a Catalyst Dx Chemistry Analyzer (IDEXX Laboratories, Inc., Westbrook, ME).

2.5. Isolation of Hepatic Total Lymphocytes. The isolation of total hepatic lymphocytes was performed as described previously [11]. In brief, mouse livers were removed and pressed through a 70 μm cell strainer. The liver cell suspension was collected and suspended in PBS, followed by centrifugation at 50 ×g for 5 min. Supernatants containing total lymphocytes were collected. The pellets were resuspended in 40% Percoll in PBS and centrifuged for 15 min at 750 ×g. 3 ml ACK Lysing Buffer was added to the tubes to lyse the residual RBCs. Then, the liver lymphocytes were washed twice with PBS and resuspended in PBS with 1% fetal bovine serum in PBS for flow cytometric analysis.

2.6. Flow Cytometry Analysis for Neutrophils. Liver lymphocytes were stained for Gr-1, CD11b, and CD62L (eBioscience, San Diego, CA, USA). Stained cells were analyzed on Cytoflex flow cytometer (Beckman Coulter, Brea, CA).

2.7. Flow Cytometric Analysis of Intracellular Reactive Oxygen Species (ROS) Production. A dihydrorhodamine 123 (DHR 123) oxidation stress assay was performed as described previously [18]. Briefly, liver lymphocytes (1×10^6) were incubated in 1 ml DMEM medium with 100 μM DHR and 1000 U/ml catalase in 37°C for 5 minutes, and 200 ng PMA was added into medium and incubated for an additional 20 minutes. Cells were washed and resuspended in PBS for flow cytometric analysis.

2.8. Real-Time Quantitative Polymerase Chain Reaction (Real-Time PCR). Total liver RNA was extracted by using the TRIzol reagent (Invitrogen, Carlsbad, CA), followed by reverse transcription into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time PCR was performed using the ABI PRISM 7500 Real-Time PCR System and SYBR Green Master Mix (Applied Biosystems) according to the manufacturer's instructions. Mouse primer sequences used are shown below:

CXCL1: Forward: TCTCCGTTACTTGGGGACAC; Reverse: CCACACTCAAGAATGGTCGC.CXCL2: Forward: TCCAGGTCAGTTAGCCTTGC; Reverse: CGGTCAAA AAGTTTGCCTTG. E-Selectin: Forward: TCTATTTCCC ACGATGCATTT; Reverse: CTGCCAAAGCCTTCAAT CAT. IL-6: Forward: ACCAGAGGAAATTTTCAATAGGC; Reverse: TGATGCACTTGCAGAAAACA.

TNFα: Forward: AGGGTCTGGGCCATAGAACT; Reverse: CCACCACGCTCTTCTGTCTAC.IL-1β: Forward: GGTCAAAGGTTTGGAAGCAG Reverse: TGTGAAATGC

CACCTTTTGA. CD14: Forward: CAGAAGCAACAGCAA CAAGC; Reverse: ACTGAAGCTTTTCTCGGAGC. 18s RNA: Forward: GGCCCTGTAATTGGAATGAGTC; Reverse: CCAAGATCCAACTACGAGCTT.

2.9. Statistical Analyses. All data are expressed as means ± SEM. Differences among groups were analyzed by one-way analysis of variance (ANOVA), and the post hoc Student-Newman-Keuls (SNK) method was used for multiple comparisons. The p value reported was two sided, and a value of $p < 0.05$ was considered statistically significant. All analyses were performed using the SPSS software (Version 12.0, SPSS Inc., USA).

3. Results

3.1. Treatment of MgIG Protected Mice from Chronic Plus Binge Ethanol Feeding-Induced Liver Injury and Steatosis. To investigate the potential hepatoprotective effects of MgIG in ALD, we used NIAAA model which can mimic major features of early ALD patients such steatosis, liver injury, and inflammation [17, 19]. MgIG was given to mice by i.p. injections daily at 22.5 mg/kg or 45 mg/kg during the course of Lieber-DeCarli ethanol liquid diet feeding and 2 hours before last ethanol gavage (Figure 1(a)). As shown in Figures 1(b) and 1(c), MgIG treatment significantly attenuated the elevation of serum ALT and AST levels induced by chronic plus binge ethanol feeding in a dose-dependent manner. It indicated that MgIG protected liver from injury caused by ethanol. In addition, MgIG greatly improved histopathological signs caused by ethanol, such as ballooning of hepatocytes and microvesicular steatosis (Figure 1(d)). Consistently, we observed a significant reduction of liver triglyceride levels in MgIG-treated mice compared with the control mice (Figure 1(e)).

3.2. Treatment of MgIG Blocked Chronic Plus Binge Ethanol Feeding-Induced Neutrophil Infiltration and Activation in the Liver. The presence of neutrophils in the liver parenchyma was a key feature of alcoholic hepatitis [5]. The infiltration of neutrophils played critical roles in the development of alcohol-induced liver damage [4, 20]. We analyzed liver neutrophils in the liver by flow cytometry. Our data confirmed that the percentages and total number of neutrophils greatly increased in the livers of chronic-binge-fed mice than in pair-fed mice in a previous report [20]. The treatment of MgIG significantly blocked the increase of both percentage of neutrophils in the liver leucocytes (Figure 2(a)). Moreover, the immunohistochemical staining of neutrophil marker myeloperoxidase (MPO) also indicated a reduction of liver neutrophils with MgIG treatment, which was consistent with the flow cytometry data (Figure 2(b)). In addition, we compared neutrophil activation marker expression by flow cytometric analysis. MgIG treatments prevent the increase of CD11b expression and the decrease of CD62L expression (Figure 2(c)), which suggested that MgIG could inhibit the activation of neutrophils in our chronic plus binge ethanol feeding model.

3.3. Treatment of MgIG Prevented Inflammatory Cytokine and Chemokine Production. To explore the mechanism on how MgIG prevented the infiltration and activation of neutrophils in the liver in the chronic plus binge ethanol feeding model, we measured several cytokines and chemokines related to the migration and activation of neutrophils in the liver with ALD. As shown in Figure 3, the mRNA expression levels of proinflammatory cytokines such as IL-6, IL-1β, and TNF-α greatly increased in the livers of chronic-binge-fed mice than in pair-fed mice. The treatment of MgIG dose dependently blocked the elevation of these cytokines. Similarly, the increase of chemokines for neutrophil migration CXCL1 and CXCL2 and adhesion molecule E-selectin was also blocked by MgIG treatment. To determine whether MgIG affected the initial response of Kupffer cells to LPS release from gut bacterial, we checked CD14 expression in the liver, as shown in Figure 3. CD14 expression significantly increased in chronic plus binge ethanol feeding mice; however, MgIG did not influence the elevation of CD14 in the liver.

3.4. MgIG Blocked Chronic Plus Binge Ethanol Feeding-Induced Neutrophil ROS Production and Oxidative Stress in the Liver. A recent study suggested that neutrophil-derived ROS and oxidative stress played important roles in alcohol-induced liver injury [18, 21]. We checked liver neutrophil ROS production by flow cytometer. As shown in Figures 4(a) and 4(b), the treatment of MgIG significantly reduced the phorbol 12-myristate 13-acetate- (PMA-) stimulated ROS levels in hepatic neutrophils. Moreover, hepatic levels of lipid peroxide including malonaldehyde (MDA) and 4-hydroxynonenal (4-HNE) were examined by immunohistochemistry. Figures 5(a) and 5(b) showed that levels of hepatic MDA and 4-HNE expression were elevated after chronic plus binge feeding, while MgIG significantly reduced hepatic MDA and 4-HNE levels in chronic plus binge mice.

4. Discussion

In our study, increased oxidative stress and neutrophil cell infiltration were observed after chronic plus binge feeding treatment. The treatment of MgIG significantly blocked the activation and infiltration of neutrophils in the chronic plus binge model. Moreover, the increased ROS generation and oxidative stress induced by ethanol were attenuated by MgIG treatment. These results suggested promising hepatoprotective effects of MgIG against ALD.

Hepatic neutrophil infiltration was considered a hall marker of alcoholic hepatitis and played critical roles in the development and progression of ALD [3, 5, 22, 23]. However, the widely used chronic Lieber-DeCarli ethanol diet feeding ALD model could only trigger very mild or no neutrophil infiltration. The recently developed chronic plus binge feeding model mimics human ALD patients drinking pattern and triggers significant liver neutrophil infiltration and liver damage. The role of neutrophils has been extensively studied by using this model. Neutrophil depletion by antibody almost completely blocked the liver injury in this model. In

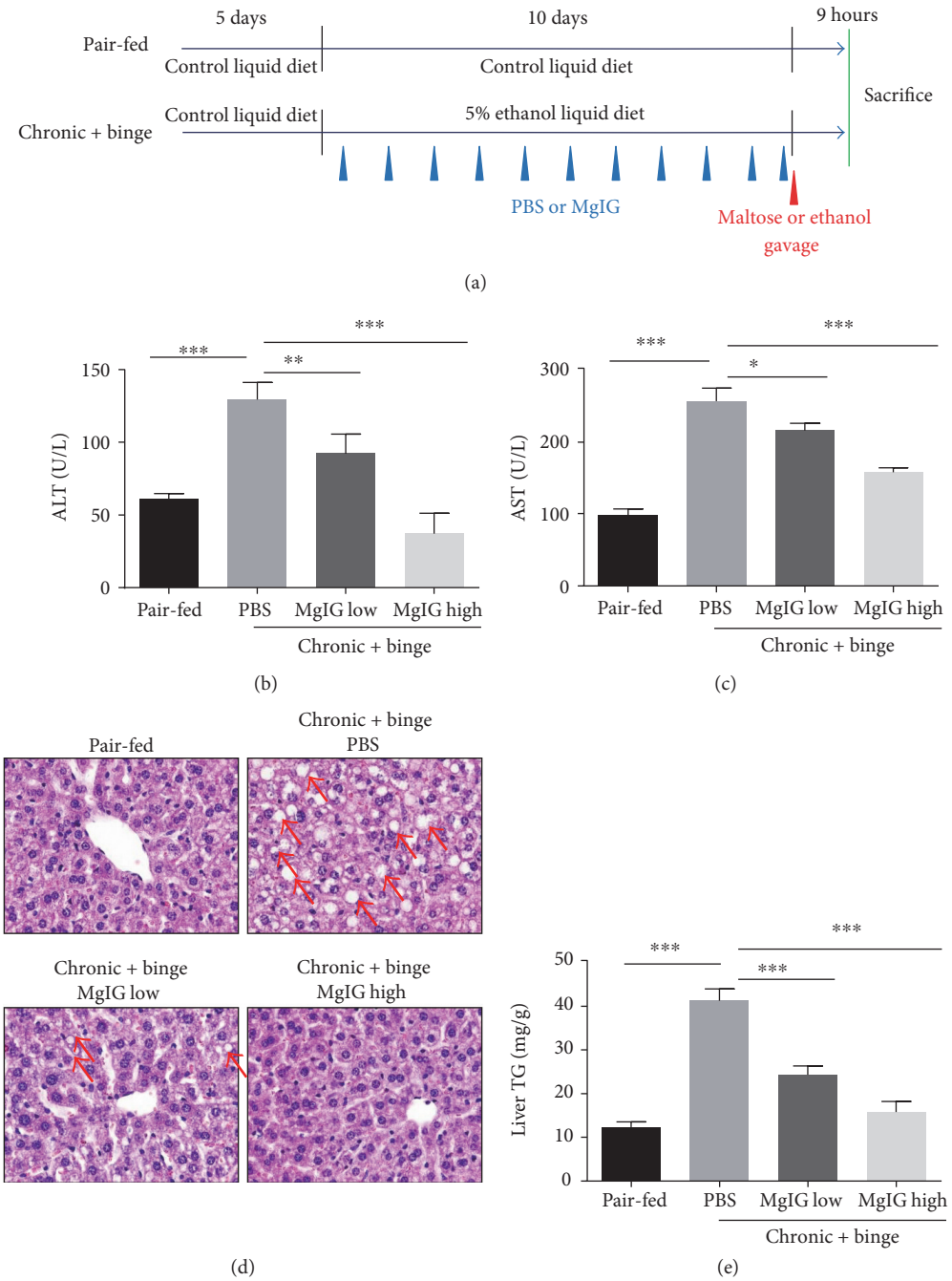

Figure 1: Protective effect of MgIG treatment against chronic plus binge ethanol feeding-induced hepatic injury. (a) Experimental design of liquid control or ethanol diet feeding and drug treatments. Mice were treated as described in (a); liver injury was assessed by measuring serum alanine aminotransferase (ALT) levels (b) and aspartate aminotransferase (AST) levels (c). (d) Representative H&E staining. Arrows indicate macrovesicular and microvesicular steatosis. (e) Hepatic triglyceride (TG) levels were measured. Values represent means ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

addition, the deficiency of E-selectin, a key adhesion molecule for neutrophil migration, greatly reduced the severity of chronic plus binge-induced liver injury [17, 20]. So, targeting neutrophil may represent an effective strategy for treating ALD. Here, we adopted the chronic plus binge feeding model to evaluate the hepatoprotective effects of MgIG on ALD and possible mechanisms involved, especially how MgIG influenced the behavior of neutrophils.

MgIG, a derivative of glycyrrhizic acid, was the extraction of the plant *Glycyrrhiza glabra*, with potential anti-inflammatory and antioxidant effects. The beneficial effects of MgIG in treating liver diseases were proven in several liver disease models including drug-induced liver damage, immune-mediated liver injury, and fatty liver. In vitro studies showed that MgIG might also reduce fat accumulation induced by ethanol [8, 10–13, 15, 24–28]. The therapeutic

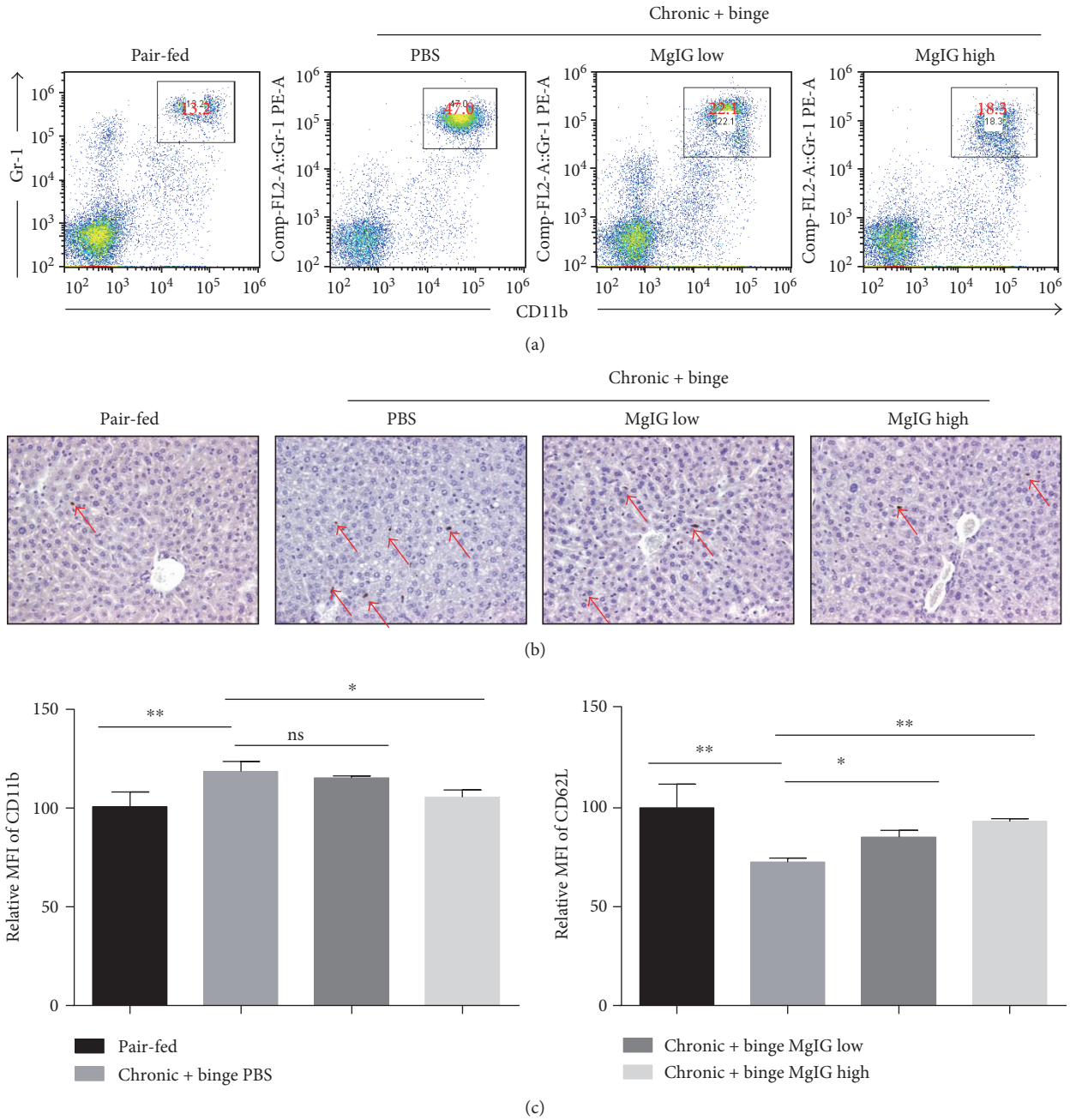

Figure 2: MgIG prevents chronic plus binge ethanol feeding-induced neutrophil infiltration in the liver. Mice were treated as described in Figure 1. (a) Liver leucocytes were isolated and analyzed by flow cytometry. The percentage of neutrophils (Gr1 + CD11b+) in liver leucocytes was determined. (b) Immunohistochemical staining of MPO-positive neutrophils in the liver. (c) Relative mean fluorescence intensity (MFI) of the cell surface levels of CD11b and CD62L on liver neutrophils determined by flow cytometry. Increase of CD11b and decrease of CD62L are associated with the activation of neutrophils. Values represent means ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$.

effect of MgIG on liver inflammatory inhibition was tested in patients with viral hepatitis, alcoholic liver disease, nonalcoholic liver disease, drug-induced liver injury, and autoimmune hepatitis by a randomized, double-blind, multi-center clinical study and prospective randomized controlled study [6]. Here, we showed that MgIG could significantly block neutrophil infiltration and activation in the chronic plus binge model. The suppression of cytokine and chemokine production in the liver was observed in MgIG-treated chronic plus binge model mice. Moreover, the production of ROS in neutrophils and liver oxidative stress was also reduced with MgIG treatment in chronic plus binge model mice. Of note, neutrophil-derived ROS has been described critical in tissue damage. So, our results supported that MgIG reduced ROS production induced by ethanol and oxidative stress in the liver. As a consequence, liver injury and subsequent liver inflammation were reduced, so that the further recruitment of neutrophils was blocked.

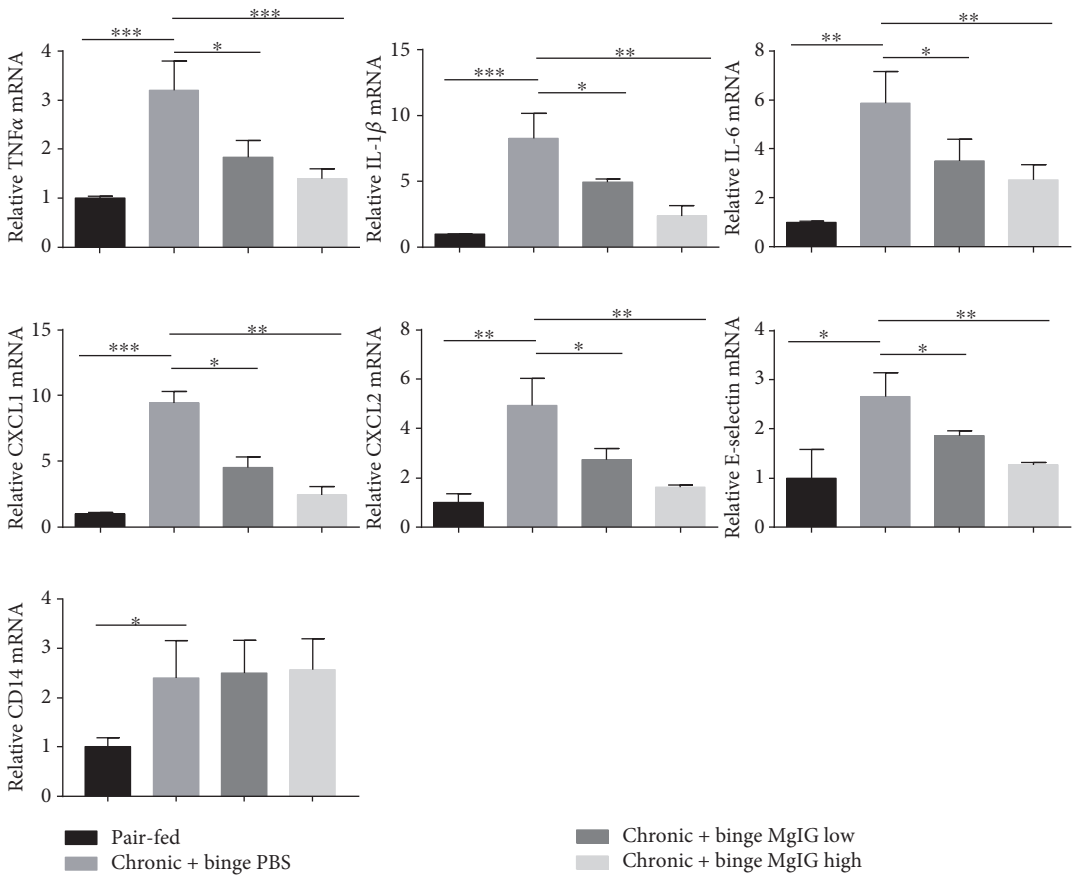

Figure 3: MgIG prevents chronic plus binge ethanol feeding-induced proinflammatory mediator expression. Gene expression of proinflammatory cytokines (TNFα, IL-1β, and IL-6), neutrophil migration-related chemokines (CXCL1 and CXCL2), neutrophil adhesion molecule (E-selection), and Kupffer cell activation marker (CD14) in the liver. Values represent means ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

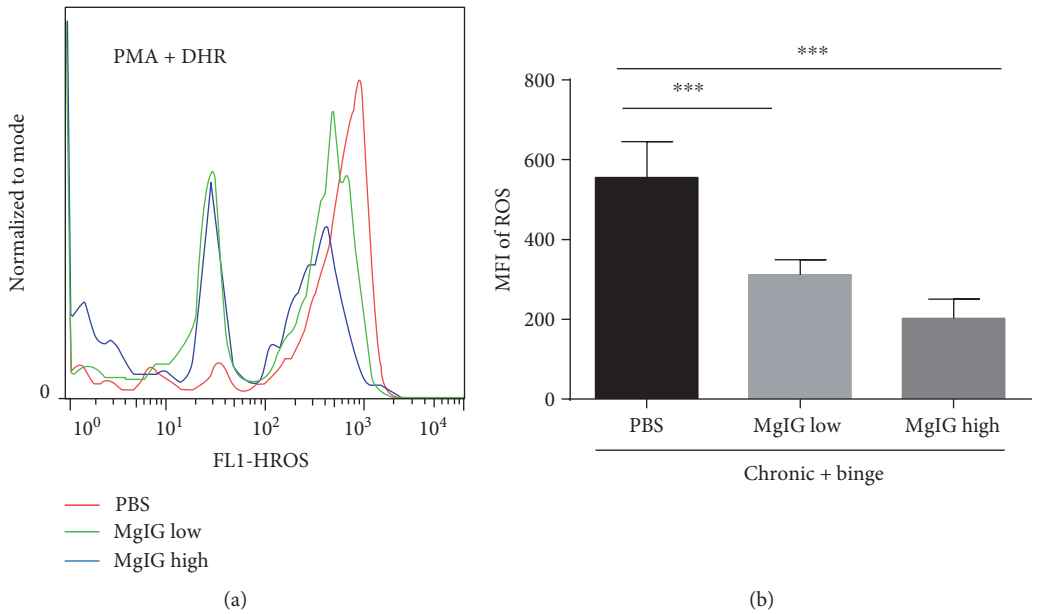

Figure 4: MgIG prevents chronic plus binge ethanol feeding-induced reactive oxygen species (ROS) production increase by liver neutrophils. (a) Liver neutrophils were isolated and stimulated with phorbol 12-myristate 13-acetate (PMA). ROS production was determined by dihydrorhodamine 123 (DHR 123) assay. (b) MFI of ROS was quantified. Values represent means ± SEM. $^{***}p < 0.001$.

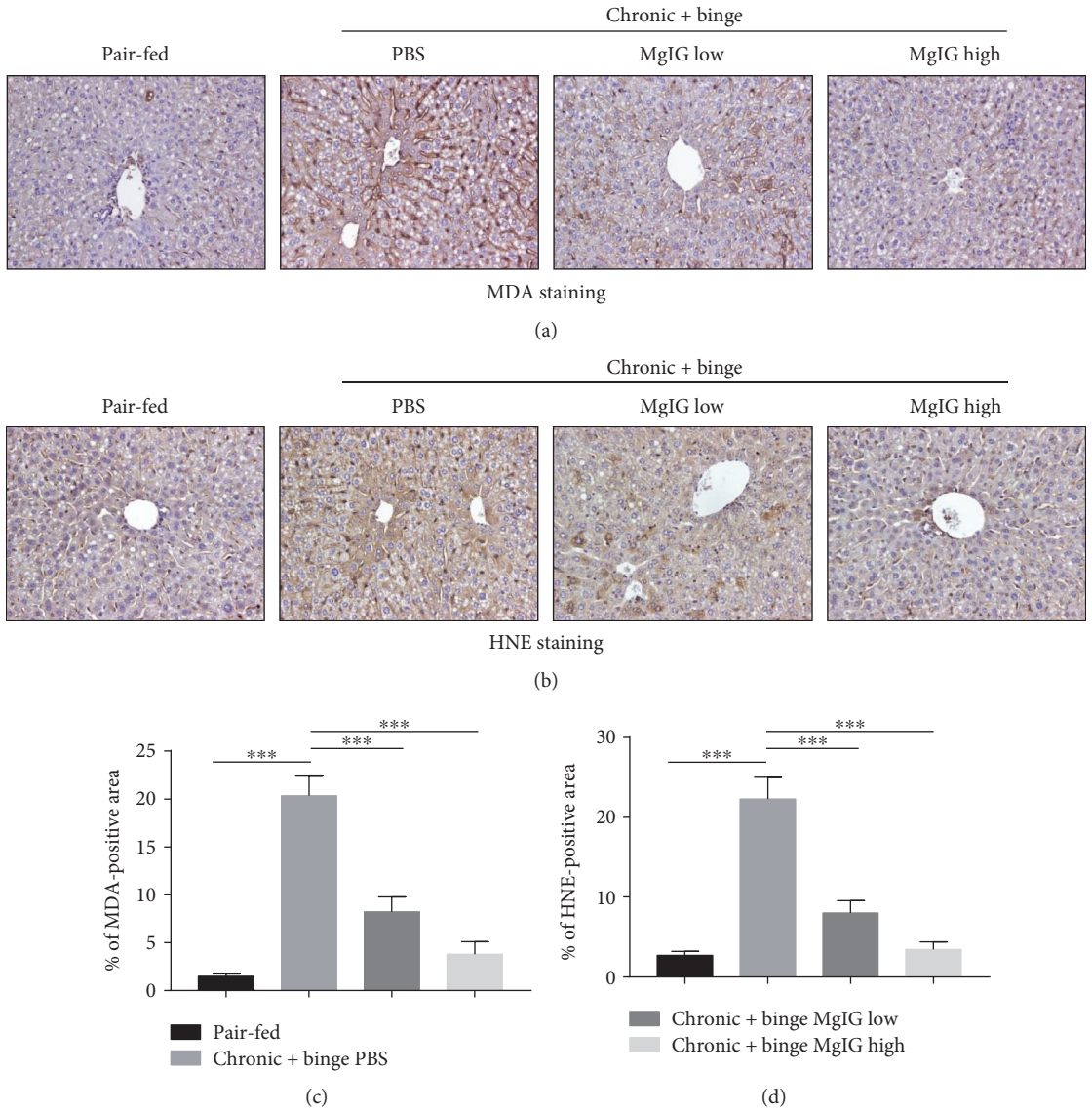

Figure 5: MgIG ameliorates chronic plus binge ethanol feeding-induced oxidative stress in the liver. Liver tissues were subjected to immunostaining with (a) an antimalonaldehyde (MDA) or (b) anti-4-hydroxynonenal (HNE) antibody. (c and d) Quantification of (a) and (b). Values represent means ± SEM. $^{***}p < 0.001$.

In summary, this study demonstrated markedly hepatoprotective effects of MgIG against chronic binge ethanol-induced liver injury. The beneficial effects may attribute reduced neutrophil ROS production, hepatic oxidative stress, and proinflammatory cytokine production. The effects of MgIG in treating ALD patients need to be evaluated in the future.

Conflicts of Interest

No conflicts of interest exist for any of the authors.

Authors' Contributions

Yan Wang and Zhenzhen Zhang are first coauthors with equal contribution.

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References

[1] J. Rehm, A. V. Samokhvalov, and K. D. Shield, "Global burden of alcoholic liver diseases," *Journal of Hepatology*, vol. 59, no. 1, pp. 160–168, 2013.

[2] L. E. Nagy, W. X. Ding, G. Cresci, P. Saikia, and V. H. Shah, "Linking pathogenic mechanisms of alcoholic liver disease with clinical phenotypes," *Gastroenterology*, vol. 150, no. 8, pp. 1756–1768, 2016.

[3] B. Gao and R. Bataller, "Alcoholic liver disease: pathogenesis and new therapeutic targets," *Gastroenterology*, vol. 141, no. 5, pp. 1572–1585, 2011.

[4] M. Dominguez, R. Miquel, J. Colmenero et al., "Hepatic expression of CXC chemokines predicts portal hypertension and survival in patients with alcoholic hepatitis," *Gastroenterology*, vol. 136, no. 5, pp. 1639–1650, 2009.

[5] H. Jaeschke, "Neutrophil-mediated tissue injury in alcoholic hepatitis," *Alcohol*, vol. 27, no. 1, pp. 23–27, 2002.

[6] Y. Yan, Y. Mo, and D. Zhang, "Magnesium isoglycyrrhizinate prevention of chemotherapy-induced liver damage during initial treatment of patients with gastrointestinal tumors," *Zhonghua Gan Zang Bing Za Zhi*, vol. 23, no. 3, pp. 204–208, 2015.

[7] Z. H. Yan, Y. M. Wang, B. Tang et al., "Meta-analysis of magnesium isoglycyrrhizinate combined with nucleoside analogues in patients with chronic hepatitis B," *Zhonghua Gan Zang Bing Za Zhi*, vol. 22, no. 2, pp. 108–112, 2014.

[8] Y. M. Mao, M. D. Zeng, Y. Chen et al., "Magnesium isoglycyrrhizinate in the treatment of chronic liver diseases: a randomized, double-blind, multi-doses, active drug controlled, multi-center study," *Zhonghua Gan Zang Bing Za Zhi*, vol. 17, no. 11, pp. 847–851, 2009.

[9] G. H. Tang, H. Y. Yang, J. C. Zhang et al., "Magnesium isoglycyrrhizinate inhibits inflammatory response through STAT3 pathway to protect remnant liver function," *World Journal of Gastroenterology*, vol. 21, no. 43, pp. 12370–12380, 2015.

[10] Q. Yang, J. Wang, R. Liu et al., "Amelioration of concanavalin A-induced autoimmune hepatitis by magnesium isoglycyrrhizinate through inhibition of $CD4^+CD25^-CD69^+$ subset proliferation," *Drug Design, Development and Therapy*, vol. 10, pp. 443–453, 2016.

[11] C. Xie, X. Li, J. Wu et al., "Anti-inflammatory activity of magnesium isoglycyrrhizinate through inhibition of phospholipase A_2/arachidonic acid pathway," *Inflammation*, vol. 38, no. 4, pp. 1639–1648, 2015.

[12] X. Huang, J. Qin, and S. Lu, "Magnesium isoglycyrrhizinate protects hepatic L02 cells from ischemia/reperfusion induced injury," *International Journal of Clinical and Experimental Pathology*, vol. 7, no. 8, pp. 4755–4764, 2014.

[13] W. Jiang, J. Liu, P. Li et al., "Magnesium isoglycyrrhizinate shows hepatoprotective effects in a cyclophosphamide-induced model of hepatic injury," *Oncotarget*, vol. 8, no. 20, pp. 33252–33264, 2017.

[14] B. Qu, R. Xing, H. Wang et al., "Multiple effects of magnesium isoglycyrrhizinate on the disposition of docetaxel in docetaxel-induced liver injury," *Xenobiotica*, vol. 47, no. 4, pp. 290–296, 2017.

[15] Q. Xu, J. Wang, F. Chen et al., "Protective role of magnesium isoglycyrrhizinate in non-alcoholic fatty liver disease and the associated molecular mechanisms," *International Journal of Molecular Medicine*, vol. 38, no. 1, pp. 275–282, 2016.

[16] C. Lu, W. Xu, J. Shao, F. Zhang, A. Chen, and S. Zheng, "Blockade of hedgehog pathway is required for the protective effects of magnesium isoglycyrrhizinate against ethanol-induced hepatocyte steatosis and apoptosis," *IUBMB Life*, vol. 69, no. 7, 2017.

[17] A. Bertola, S. Mathews, S. H. Ki, H. Wang, and B. Gao, "Mouse model of chronic and binge ethanol feeding (the NIAAA model)," *Nature Protocols*, vol. 8, no. 3, pp. 627–637, 2013.

[18] M. Li, Y. He, Z. Zhou et al., "MicroRNA-223 ameliorates alcoholic liver injury by inhibiting the IL-6-p47phox-oxidative stress pathway in neutrophils," *Gut*, vol. 66, no. 4, pp. 705–715, 2017.

[19] S. H. Ki, O. Park, M. Zheng et al., "Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3," *Hepatology*, vol. 52, no. 4, pp. 1291–1300, 2010.

[20] A. Bertola, O. Park, and B. Gao, "Chronic plus binge ethanol feeding synergistically induces neutrophil infiltration and liver injury in mice: a critical role for E-selectin," *Hepatology*, vol. 58, no. 5, pp. 1814–1823, 2013.

[21] Z. V. Varga, C. Matyas, K. Erdelyi et al., "Beta-Caryophyllene protects against alcoholic steatohepatitis by attenuating inflammation and metabolic dysregulation in mice," *British Journal of Pharmacology*, 2017.

[22] S. Das, J. S. Maras, M. S. Hussain et al., "Hyperoxidized albumin modulates neutrophils to induce oxidative stress and inflammation in severe alcoholic hepatitis," *Hepatology*, vol. 65, no. 2, pp. 631–646, 2017.

[23] B. Gao and M. Xu, "Chemokines and alcoholic hepatitis: are chemokines good therapeutic targets?," *Gut*, vol. 63, no. 11, pp. 1683-1684, 2014.

[24] W. H. Chen, L. G. Lu, M. D. Zeng et al., "Effect of magnesium isoglycyrrhizinate on the proliferation and oxidative stress of rat hepatic stellate cells in vitro," *Zhonghua Gan Zang Bing Za Zhi*, vol. 14, no. 6, pp. 426–430, 2006.

[25] Y. Cheng, J. Zhang, J. Shang, and L. Zhang, "Prevention of free fatty acid-induced hepatic lipotoxicity in HepG2 cells by magnesium isoglycyrrhizinate in vitro," *Pharmacology*, vol. 84, no. 3, pp. 183–190, 2009.

[26] J. Jin, J. M. Xu, X. C. Liu, and Q. Mei, "Effect of magnesium isoglycyrrhizinate on concanavalin A (Con A)-induced immunological liver injury in mice," *Zhonghua Gan Zang Bing Za Zhi*, vol. 17, no. 5, pp. 389-390, 2009.

[27] B. Vincenzi, G. Armento, M. Spalato Ceruso et al., "Drug-induced hepatotoxicity in cancer patients - implication for treatment," *Expert Opinion on Drug Safety*, vol. 15, no. 9, pp. 1219–1238, 2016.

[28] Z. Zhao, Z. Tang, W. Zhang, J. Liu, and B. Li, "Magnesium isoglycyrrhizinate protects against renalischemiareperfusion injury in a rat model via antiinflammation, antioxidation and antiapoptosis," *Molecular Medicine Reports*, vol. 16, no. 3, pp. 3627–3633, 2017.

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Research Article

Serum Cytokeratin 18 M30 Levels in Chronic Hepatitis B Reflect Both Phase and Histological Activities of Disease

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Chronic hepatitis B has highly a dynamic course with significant fluctuations of HBV-DNA and ALT impeding assessment of disease activity. New biomarkers of inflammatory versus noninflammatory stages of HBV infection are urgently needed. Cytokeratin 18 epitope M30 (M30 CK-18) is a sensitive marker of cell death. We aimed to investigate an association between serum M30 CK-18 and histological activity and phase of HBV infection. 150 Caucasian patients with HBV-infection were included in the study. Serum M30 CK-18 levels reflected phase of disease, being significantly higher in both HBeAg(+) and HBeAg(−) hepatitis B in comparison to HBsAg(+) carrier groups. The highest serum M30 CK-18 levels were observed in subjects with the most advanced stages of HBV. Moreover, its serum concentrations correlated with both inflammatory activity and fibrosis advancement (ANOVA $P < 0.001$). Importantly, serum M30 CK-18 levels were able to discriminate patients with mild versus moderate-advanced fibrosis (AUC: 0.86) and mild versus active liver inflammation (AUC: 0.79). M30 CK-18 serum concentration has good sensitivity and specificity in discriminating mild versus moderate/severe fibrosis and inflammation even in patients with normal ALT activity. This study suggests M30 CK-18 as a potential noninvasive marker of disease activity and also a marker of phase of persistent HBV infection.

1. Introduction

Persistent HBV infection, especially following HBeAg seroconversion, is a highly dynamic disease with significant fluctuations of HBV-DNA which may be observed in every phase of disease [1]. This variability is a result of a complex interplay between viral factors, mainly quantity and transcriptional activity of cccDNA, and strength of HBV-specific immune responses. It is important to note that frequent exacerbations of chronic hepatitis B (CHB) results in the progression of liver fibrosis which eventually leads to liver cirrhosis [2]. The commonly used marker of inflammatory activity, ALT, has significant limitations in HBeAg(−) hepatitis. It has been shown that as many as 44% of HBeAg(−) hepatitis ALT remain within normal range for the majority of time despite of disease progression. Importantly, its increase could only be observed once a year in almost 60% of those patients [3]. Moreover, ALT activity does not reflect inflammatory activity in the liver nor extent of fibrosis in CHB. The presence of biopsy-proven histologic damage (necroinflammation and fibrosis) is common even when ALT is less than 2XULN in patients with chronic hepatitis B (CHB) [4]. Liver biopsy has been a gold standard of viral hepatitis activity; however, this invasive procedure is associated with risk of complications and yields variability due to the uneven distribution of lesions [5]. Liver biopsy examination shows interpathologist variations ranging from 10 to 20% [6]. Finally, novel noninvasive modalities including elastography show limited capability of differentiating between mild and moderate fibrosis, which is of importance for the selection of anti-HBV therapy candidates [7]. A potential advantage of serum noninvasive markers of liver fibrosis is that total liver fibrosis

Table 1: Characteristics of studied population (median, IQR). Significant differences between consecutive phases by Kruskall-Wallis ANOVA were marked as follows: $^{*}P$ value < 0.05 and $^{***}P < 0.005$.

	Total group ($n = 150$)	HBeAg(+) hepatitis ($n = 10$)	Low replicative carriers ($n = 47$)	High replicative carriers ($n = 28$)	HBeAg(−) hepatitis ($n = 65$)
Gender, M *n* (%)	86 (57)	7 (70)	19 (40)	15 (53)	45 (69)
Age, years	33 (23–42)	39 (24–53)	34 (25–45)	30 (23–39)	32 (23–40)
HBeAg(+), *n* (%)	10 (7)	10 (100)	—	—	—
HBV-DNA log10 IU/mL	4.1 (2.5–5.1)	5.1 (4.7-8.0)	2.3 (2.3–2.4)	3.9 (3.5–4.3)	5.0 (4.4-5.7)***
HBsAg log10 IU/mL	4.2 (3.6–4.6)	4.3 (3.9–5.0)	4.1 (3.3-4.6)	4.1 (3.6–4.6)	4.3 (4.1–4.6)
ALT, IU/mL	30 (22–44)	70 (43–88)	23 (18–31)	27 (23–33)	36 (26–73)***
GGT, IU/mL	18 (11–32)	28 (22–41)	13 (10–20)	18 (13–21)	25 (12–47)***
Platelets, 10^9/L	190 (157–220)	122 (110–154)	189 (155–219)	196 (171–224)	193 (166–219)
Prothrombin ratio, %	100 (90–107)	89 (82–98)	103 (96–110)	102 (92–110)	96 (89–107)*

is reflected consequently reducing the risk of intra- and inter-assay variability which is known for liver biopsy and liver elastography. Therefore, there is a strong need for new noninvasive markers of liver injury, especially detecting inflammatory activity not only fibrosis, particularly at less advanced stages. Knowing that cell death by apoptosis is an important step for the development of CHB, the integration of markers of cell death appears to be justified [8, 9].

Apoptosis is a major cause of hepatocytes' death in chronic viral hepatitis. Apoptosis leads to the activation of several cysteine-aspartate proteases, called caspases. Caspases cleave cellular proteins, including cytokeratin-18 (CK-18). CK-18 is an intermediate filament protein of the cytoskeleton which may be found in epithelial cells, especially of the digestive tract. Along with cytokeratin 8, CK-18 is the only cytokeratin found in the hepatocytes [10]. Recently, the evaluation of CK 18 has been validated as a marker of inflammation activity and fibrosis in chronic hepatitis C (CHC) and nonalcoholic fatty liver disease (NAFLD) [11–14]. During apoptosis, activated caspases 3, 6, 7, and 9 are able to cleave cytokeratin-18 at specific peptide recognition sites [12, 15]. As a result of caspase cleavage, cytokeratin-18 is cut at position 387 to 396 and releases its fragment M30. Epitope M30 is released into the bloodstream as a result of cell death; therefore, it is possible to use M30 CK-18 as a circulating biomarker of epithelium apoptosis [14, 16]. Previously, Papatheodoridis et al. [17] demonstrated significantly higher concentrations of M30 CK-18 in patients with active CHC-B compared to nonactive carriers with low HBV replication. Of importance, serum concentration of CK-18 correlated with HBV replication in contrast to chronic hepatitis C. There was also a significantly higher concentration of M30 CK-18 in patients with CHC-B and normal ALT activity compared with HBsAg carriers [17, 18].

Another recent marker in CHB is serum HBsAg quantification which reflects the phase of HBV infection [19]. In HBV-genotype D, the combined single-time measurement of HBV-DNA < 2000 IU/mL and HBsAg < 1000 IU/mL had excellent diagnostic accuracy in discriminating active and inactive HBV persistent infection in the long-term follow-up [20]. More recent data suggested that HBV genotype should be included as another factor influencing HBsAg levels in the natural course of CHB and the correlation between phase of disease and HBsAg levels might be weak/not present in HBV-A. Importantly, an association between HBsAg and liver fibrosis stage has been mainly shown in patients with HBeAg(+) disease and its significance decreases in liver cirrhosis, especially in HBeAg(−) [21].

The aim of study was to evaluate the clinical usefulness of serum M30 CK-18 in the noninvasive assessment of phase of disease, inflammatory activity, and liver fibrosis in the natural course of chronic hepatitis B in predominantly HBV genotype A infected population.

2. Patients and Methods

150 Caucasian patients with persistent HBV infection (median age 33, 86 males) were included in this single-center, cross-sectional study. All patients were adults and had HBsAg(+) for at least 6 months. The clinical characteristics of the studied population is presented in Table 1. HBeAg-negative subjects were further stratified into low replicative carriers (LRC: normal ALT-activity and HBV-DNA < 2000 IU/mL), high replicative carriers (HRC: HBV-DNA between 2000 and 20,000 IU/mL, normal ALT (N ALT) and/or no inflammatory lesions in liver biopsy), and HBeAg-negative hepatitis (ENH: HBV-DNA > 20,000 IU/mL and increased ALT and/or inflammatory lesions in liver biopsy). Liver biopsies were performed only in patients with HBV-DNA ≥ 2000 IU/mL and clinical suspicion of active hepatitis B, as a part of the qualification for the anti-HBV therapy. The results of liver biopsy were available in 66 (44%) patients. Exclusion criteria included coinfection with HCV, HIV, liver steatosis, autoimmune

disorders, malignancies, and current alcohol abuse. The study protocol was approved by the Bioethics Committee of Medical University in Bialystok, and informed consent was obtained from each participant.

2.1. Liver Morphology. Liver tissue was collected from the right liver lobe, using the Menghini method with a disposable set of Hepafix Luer Lock (Braun) 16G needle. Tissue was fixed with 4% formalin and then paraffin embedded. Biopsies' length was in the range of 10–30 mm. Samples sections were stained with: hematoxylin-eosin to identify liver inflammation and Sirius Red for fibrosis assessment. Liver biopsies from CHB patients were scored by a blinded pathologist using 5-category (grading of 0–4 and staging of 0–4) Scheuer classification.

2.2. Serum M30 CK-18 Measurement. A total volume of 10 mL peripheral venous blood was taken at the time of liver biopsy and/or liver function test assessment and stored at −80°C until further processing. Serum M30 CK-18 was measured by M30-Apoptosense ELISA assay (PEVIVA AB, Bromma, Sweden) according to the manufacturer's recommendations. Lower limit of detection and intra- and interassay variability were (25 U/L (8.44 pg/mL), <10%, resp.).

2.3. Serum HBsAg and HBV-DNA Quantification. Serum HBsAg levels were measured using the Abbott ARCHITECT® assay (Abbott Diagnostics, Abbott Park, IL). Dynamic range of this test was 0.05–250 IU/mL. Dilution of samples was 1 : 20, 1 : 100, and 1 : 500, respectively. HBsAg result less than 0.05 IU/mL was measured without dilution. HBsAg quantification is expressed in IU/mL. Serum HBV-DNA was measured using COBAS AmpliPrep/COBAS TaqMan (Roche Diagnostics, Mannheim, Germany) and TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA) with detection limits of 12 IU/mL and 50 IU/mL, respectively. Results are given in IU/mL. HBsAg and HBV-DNA values were log-transformed.

2.4. Statistical Analyses. Data is presented as median (IQR) and percentage when appropriate. Nonparametric, distribution-free tests were applied. Differences between groups were analyzed by Mann–Whitney U test and the Kruskall-Wallis ANOVA test. Correlation analyses were performed by Spearman's test. In order to determine the diagnostic accuracy (sensitivity and specificity) of M30 CK-18, receiver operating characteristic (ROC) curves were drawn and the area under the curve (AUC) was calculated. A $P < 0.05$ was considered statistically significant. Statistical analyses were performed by the GraphPad Prism5 (La Jolla, CA) and Statistica 10.0 (Statsoft, Tulsa, OK).

3. Results

In this Caucasian treatment-naïve cohort, among the 150 subjects included, 93% were HBeAg-negative and 69% had normal ALT activity. Among the HBeAg(−) patients, 47 were low replicative carriers (LRC), 28 high replicative carriers (HRC), and 65 HBeAg(−) hepatitis subjects (ENH). Importantly, among the ENH patients with available liver biopsies in 11 (28%), ALT activity was within normal range although active inflammatory lesions (G2 or more) were shown in the liver biopsy (Table 1).

Table 2: Correlations between serum M30 CK-18 (U/L) and liver function tests as well as HBV-DNA and HBsAg levels (R value by Spearman's rank test; β value by multiple regression; $^*P$ value < 0.05).

Parameter	R value	P value	Multiple regression β value, P value
Age, years	0.13	0.09	—
HBV-DNA, log10 IU/mL	0.26	0.02*	$B = 0.23$, $P = 0.05$*
HBsAg, log10 IU/mL	−0.04	0.6	—
ALT, IU/mL	0.28	0.004*	$B = 0.32$, $P = 0.002$*
GGT, IU/mL	0.24	0.005*	$B = 0.25$, $P = 0.016$*
Platelets, 10^9/L	−0.18	0.05*	$B = -0.06$, $P = 0.41$

3.1. M30 CK-18 and Biochemical Activity and Phase of Persistent HBV Infection. Serum M30 CK-18 correlated with ALT ($R = 0.28$, $P = 0.004$), GGT activity ($R = 0.24$, $P = 0.005$), and platelet count ($R = -0.18$, $P = 0.05$) (Table 2). Subjects with increased ALT had significantly higher serum M30 CK-18 compared to the group with normal ALT (267 (129–567) versus 163 (109–244) U/L, $P = 0.001$). Moreover, M30 CK-18 showed a significant association with HBV-DNA ($R = 0.26$, $P = 0.02$), which was further proved in multivariate regression (Table 2). Serum M30 CK-18 levels showed an association with phase of disease. The lowest values were observed in inactive CHB (LRC: 156 (81–208) IU/mL; HRC: 177 (78–261) IU/mL), while M30 CK-18 was significantly higher in both active hepatitis B groups (ENH: 225 (141–438) IU/mL; HBeAg(+) hepatitis 532 (367–759) IU/mL), Figure 1.

3.2. M30 CK-18 and Histological Activity of Hepatitis. Serum M30 CK-18 levels were highly associated with the histological advancement of liver fibrosis (ANOVA, $P < 0.0001$) and the degree of inflammation (ANOVA, $P = 0.0009$) (Figure 2, Table 3). Serum M30 CK-18 was more than trifold higher in patients with moderate/severe (S2–S4) versus mild (S1) fibrosis (534 (272–1345) versus 119 (67–207) IU/mL, $P < 0.0001$) (Table 3). To determine the diagnostic potential of the M30 CK-18, ROC analysis was performed. ROC showed good a discriminatory ratio for patients with moderate/severe versus mild fibrosis (AUC: 0.86, $P < 0.0001$), with 84% sensitivity and 80% specificity for M30 CK-18 value of 253 IU/mL (Figure 3). Similarly, M30 CK-18 was significantly higher in subjects with active inflammation (G1-G2: 124 (74–229) versus G3-G4: 466 (229–1307) IU/mL, $P < 0.001$) with AUC of 0.79 (Figure 2).

3.3. HBsAg Levels and the Phase of Persistent HBV Infection. As reported previously, HBsAg serum levels showed only moderate correlation with HBV-DNA ($R = 0.17$, $P = 0.03$) and were not associated with ALT or fibrosis stage in the natural history of HBeAg(−) persistent HBV infection [19]. The

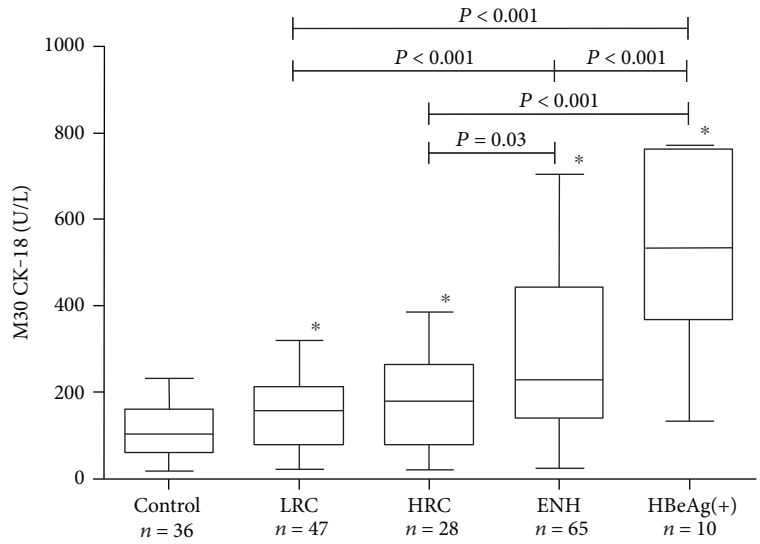

Figure 1: Median (IQR) serum M30 CK-18 levels in different phases of persistent HBV infection (LRC: low replicative carriers; HRC: high replicative carriers; ENH: HbeAg(−) hepatitis B; HBeAg(+): HBeAg(+) immune clearance phase). $^*P < 0.05$ in comparison with the control group. All comparisons by use of Mann–Whitney U test.

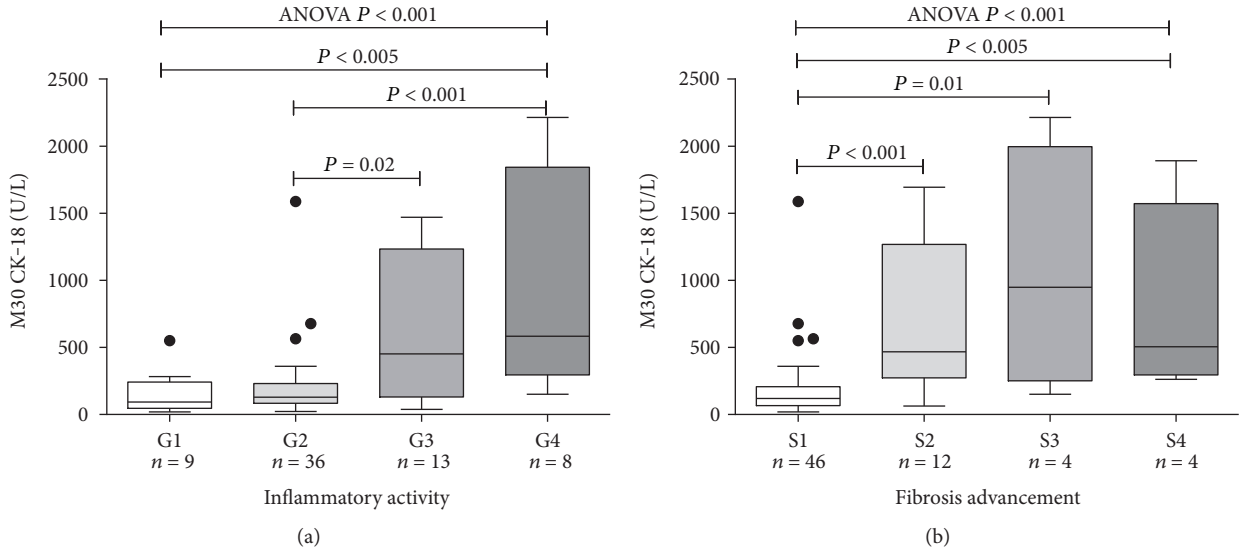

Figure 2: Median (25–75% CI) serum M30 CK-18 (U/L) concentration in HBV-infected patients with subsequent grades of inflammatory activity (a) and stages of liver fibrosis (b) in the liver histology. Comparisons by use of Mann–Whitney U test and Kruskal-Wallis ANOVA.

highest concentrations of serum HBsAg were observed in both phases of active hepatitis with positive HBeAg (4259 (3898–4960) log10 IU/mL) and negative HBeAg (4252 (4077–4618) log10 IU/mL). Patients with low disease activity (HBsAg carriers) had lower HBsAg concentrations (HRC: 4093 (3623–4631) log10 IU/mL; LRC: 4085 (3277–4598) log10 IU/mL), with statistically significant difference between ENH and low replicative HBsAg carriers ($P = 0.03$). Serum CK-18M30 did not show an association with HBsAg concentration ($R = 0.04$, $P = 0.61$), which suggests an additional advantage of M30 CK-18 measurement in addition to HBsAg quantification.

4. Discussion

In this study, we evaluated an association between serum M30 CK-18 and biochemical and histological activities of chronic hepatitis B. Establishing a more precise marker of both disease activity and fibrosis in CHB currently unmet the medical needs, especially in HBeAg(−) hepatitis which is characterized by significant variations of ALT and HBV-DNA. Such noninvasive biomarker would allow precise dissection between inflammatory and noninflammatory stages of CHB but also selection of best candidates for PEG-IFN and novel immunomodulatory therapies [22].

Table 3: Serum M30 CK-18 (median ± IQR) concentrations with regard to inflammatory activity and fibrosis stage in liver biopsy.

	Inflammatory activity		Fibrosis advancement	
	G0–G2 ($n = 45$)	G3–G4 ($n = 21$)	S0–S1 ($n = 46$)	S2–S4 ($n = 20$)
Age, years	31 (25–38)	39 (21–59)	31 (25–38)	39 (23–51)
HBV-DNA, log10 IU/mL	4.46 (3.6–5.28)	4.00 (3.23–6.19)	4.46 (3.61–5.27)	4.00 (3.18–7.39)
HBsAg, log10 IU/mL	4.25 (3.94–4.59)	4.14 (3.95–4.77)	4.27 (3.99–4.61)	4.11 (3.95–4.57)
CK-18, U/L	124 (74–225)	466 (197–1268)	120 (74–201)	521 (272–1268)
ALT, IU/mL	34 (26–52)	73 (30–109)	34 (27–51)	73 (36–109)
GGT, IU/mL	19 (12–32)	38 (28–114)	19 (12–32)	38 (28–114)
Platelets, 10^9/L	198 (170–219)	127 (107–201)	201 (170–219)	126 (107–164)

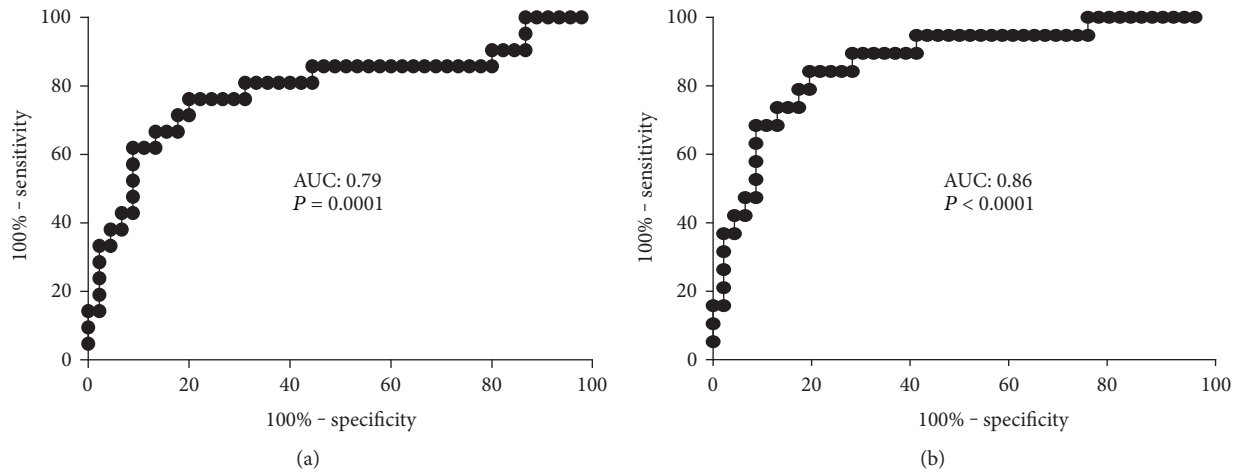

Figure 3: ROC curves for serum M30 CK-18 concentrations as a marker of (a) active liver inflammation (G1-G2 versus G3-G4) and (b) advanced fibrosis (S0-S1 versus S2–S4). AUC: area under curve.

Previous studies on M30 CK-18 have generally focused on CHC and NAFLD [14]. Increase in M30 activity has been shown in patients with CHC [13, 17, 23]. Bantel et al. [13] proved that 27% of patients with CHC normal level of aminotransferases occurred despite developing liver damage. Moreover, the authors found higher concentrations of M30 CK-18 in patients with advanced fibrosis and suggested that M30 might be more sensitive than aminotransferase for identifying liver injury in CHC [13]. In another study by Papatheodoridis, a positive correlation between serum CK-18 and severity of inflammatory activity and fibrosis was found in chronic HCV infection. In this study, the concentration of CK-18 exceeding 225 U/L demonstrated a positive and negative predictive values for moderate–severe histologic lesions in the liver biopsy of 70% and 74% [18]. Similar findings by Parfieniuk-Kowerda et al. have been shown in our center [24]. The ROC analysis revealed that serum M30 CK-18 showed 75% sensitivity and 75% specificity in differentiating between mild, moderate, and severe inflammation at a concentration of >204 U/L, whereas higher concentration (>330 U/L) achieved 89% sensitivity and 78% specificity between mild and moderate to advanced liver fibrosis in chronic hepatitis C [24].

Likewise, in NAFLD, serum M30 CK-18 was shown to be elevated and strongly associated with ALT and AST activities. A negative correlation between the level of M30 and AST/ALT ratio was observed suggesting that apoptosis was more a dominant mechanism of cell death [25]. In another study, a novel algorithm for assessing fibrosis in NAFLD was suggested, including ALT, AST, M30, M60, and HA. Importantly, the authors suggested M30 and M65 as more important for the decision than the classic liver parameters [9]. In further study in NAFLD, apoptosis-specific M30 CK-18 correlated with reticuloendothelial system (RES) cell iron in the liver and nonalcoholic steatohepatitis [10]. In their recent study, In their recent study, Bantel et al. applied an improved ELISA for serum cytokeratin-18 fragment detection suggesting the use of the method to evaluate the early stages of development of NASH; it also enables distinguishing differences between patients with a minimal (≤10%) and advanced (>10%) hepatic steatosis [26].

There is limited information concerning M30 CK-18 levels in CHB patients. Compared with conventional indicators of the activity of liver disease, determination of caspase activity, expressed as the concentration of CK-18 in the serum, may be a more sensitive method for assessing the activity of persistent HBV infection. The results of our study show that there is a significant difference in the concentration of M30 CK-18 between consecutive phases of CHB including HBeAg (+), LRC, HRC, and ENH (ANOVA, $P < 0.0001$). Interestingly, this marker was able to differentiate patients with active HBeAg-negative hepatitis B and low replicative

and high replicative carriers. It is most likely a consequence of association between M30 CK-18 and ALT activity but also with HBV-DNA found in our study. A positive correlation between serum M30 CK-18 and ALT-activity was found in all previous studies in CHB [14, 17, 27], while with HBV-DNA, only in some [17]. This might be the effect of study group composition where all phases of CHB should be present to allow appropriate comparisons. Importantly, also site-specific phosphorylation of K18 correlated with the elevation of both histological lesions and enzymatic activities of alanine aminotransferase in CHB which further supports biochemical data [28].

Definitely in the era of noninvasive methods of activity assessment, the most important is the association between serum M30 CK-18 and histologic advancement of lesions in the liver in CHB. We have demonstrated that serum concentrations of M30 CK-18 were associated with histological inflammatory activity (ANOVA, $P = 0.0009$) and advancement of liver fibrosis (ANOVA, $P < 0.0001$). It is important since majority of the previously available noninvasive tests, including transient elastography, are not able to differentiate between the degrees of inflammation in CHB, especially in the case of the less advanced liver damage. ROC curve analysis showed that the single measurement of the concentration of M30 CK-18 > 253 U/L differentiates patients with mild to moderate versus active hepatitis with 80% specificity and 76% sensitivity. Furthermore, M30 CK-18 with the same threshold exhibited 84% sensitivity and 80% specificity in differentiating patients with mild versus advanced fibrosis (S1 versus S2–S4). Naturally, restricted number of patients with histological evaluation of liver inflammation and fibrosis is a limitation of the study. Liver biopsies, as an invasive procedure, were only performed in subjects with significant HBV viral load. On the other hand, even in this limited sample study, statistical results suggest for a highly significant association between serum M30 CK-18 and inflammatory activity as well fibrosis stage. In the previous study, Sumer et al. reported [14] that the M30 CK-18 levels are the highest in patients with liver cirrhosis. Also, Bae et al. [27] found that serum M30 levels are associated with the presence of significant inflammation. In our study, by means of serum M30 CK-18, the differentiation even between mild (S1) and moderate–advanced (S2–S4) fibrosis was possible. This marker could be especially useful in subjects with normal ALT and slightly elevated HBV-DNA as well as in HBeAg(+) highly vireamic patients with normal ALT in which otherwise long-term follow-up or liver biopsy would be necessary in order to assess the activity of the disease.

Clinical usefulness of serum M30 CK-18 could potentially be further increased by combining it with already established serological markers of hepatitis B, including HBsAg quantification. The concentration of HBsAg in serum reflects the transcriptional activity of the cccDNA and the degree of integration of HBV into the host genome [29]. In our previous study including 226 Caucasian patients with chronic hepatitis B, HBsAg levels showed significant differences during the natural course of HBV infection. Low HBsAg levels were characteristics of inactive carriers and differentiated this group from HBeAg(−) hepatitis B patients with normal ALT activity and fluctuating HBV-DNA [19]. This finding was further confirmed by Brunetto et al. who showed that single time-point, combined measurement of HBsAg < 1000 IU/mL and HBV-DNA < 2000 IU/mL identified inactive HBsAg carriers with 94.3% diagnostic accuracy in HBV genotype D infection [20]. Importantly, HBsAg levels and on-therapy dynamics of decline depends on HBV genotype which has to be taken into account. In the current study, again, we showed higher HBsAg concentrations in patients with HBeAg-negative hepatitis compared to patients with low replication (4.252 versus 4.085 log10 IU/mL, $P = 0.04$). The difference was less pronounced than in the previous study [19] which most likely results from high concentrations of HBsAg observed in the serum of patients infected with genotype A, which dominates the population of Poland (>70%) [30]. There was no correlation between serum HBsAg and M30 CK-18 levels, which was expected since HBsAg levels do not correlate with inflammatory activity in CHB. On the other hand, the combined measurement of those two markers, M30 CK-18 (inflammatory activity and fibrosis) and HBsAg-levels (cccDNA activity and phase of disease), could deliver precise characteristics of CHB activity.

Majority of HCC cases are related to chronically infected HCV (75–80%) and HBV (10–15%). An important risk factor for HCC is cirrhosis of the liver (80–90% of HCC cases) [31]. Recent studies have shown that elevated M30 CK-18 concentrations may be a useful marker for early stages HCC. Elalfy et al. calculated a ROC curve for the M30 CK-18 to discriminate between macrovascular invasions of HCC, which has shown 100% sensitivity and 98% specificity for a cutoff of 304.5 ng/mL (AUC: 0.997, $P < 0.001$) [32].

For noninvasive evaluation of fibrosis and necroinflammatory activity, several serum biomarkers can be applied, including Fibrotest and Actitest, Hepascore MPP. Ngo et al. [33] in a large group of 1300 patients with CHB showed higher Fibrotest prognostic value for ALT in predicting disease progression [33]. Fibrotest and Actitest and HBV-DNA combinations have been shown to better separation patients with low HBV replication as compared to the measurement ALT or HBV-DNA alone [33]. Despite the large study group (1300 patients), comparing Fibrotest and Actitest of a liver biopsy was performed only in the group of 97 patients. Hepascore is another test evaluation for histological changes in the liver. The comparison of Hepascore to Fibrotest shows a similar diagnostic value with high AUC (area under the curve) values for significant fibrosis, advanced fibrosis, and cirrhosis [34]. The abovementioned studies were designed primarily to assess the degree of fibrosis in hepatitis C, and their utility in evaluating fibrosis in CHB was often not validated. Another study which noninvasively assessed fibrosis shows that the overall rate was significantly lower in patients with CHB compared to patients with CHC in the early stages of fibrosis ($F \leq 2$) [8]. It is also important to underline that panels of biomarkers like Fibrotest and Actitest which are composed of several complex measures (e.g., alpha-2 macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, ALT, and age) and are also patented which increases their costs in routine clinical practices.

Another noninvasive marker of liver fibrosis may be MMPs which are the main enzyme-degrading proteins of extracellular matrix and play an important role in tissue reconstruction and repair in physiological and pathological conditions. Previous studies have shown that serum MMP-2 levels were statistically higher in CHB patients compared to controls ($P = 0.001$) [14]. Researchers have shown that M30 CK-18 and MMP-2 concentrations were higher in patients with CHB compared to healthy controls ($P = 0.001$ and $P = 0.001$, resp.) and were associated with significant liver fibrosis. What is worth emphasizing is that the assessment of MMP-2 levels did not show the difference between stages 1 and 2 as well stages 2 and 3 of liver fibrosis, highlighting the importance of labeling M30 CK-18 as a more sensitive marker than MMP-2 in predicting liver fibrosis [14]. In our study, we have shown not only the importance of the role of M30 CK-18 as a fibrosis marker but also the importance of M30 CK-18 as a good marker for inflammatory activity, which is of clinical importance for anti-HBV therapy indication, for example, in patients with high activity but with low fibrosis.

It is worth emphasizing that the determination of serum M30 CK-18 is more selective for the measurement of total CK-18 concentration. The measurement of the M30 CK-18 fragment reflects the hepatocytes apoptosis, which is a key process for the removal of infected cells in CHB. There are also other CK-18 fragments, such as the M65 epitope. In the study of Joka et al. [35], it was found that M65 was an additional indicator of a complementary M30, since it reflects the processes of necrosis. Reis et al. [36] in a study involving 76 patients with CHC after OLTx (orthotopic liver transplantation) measured M30S, M30H CK-18, and M65 CK-18 concentrations and found that these markers were able to discriminate between acute reinfection and acute transplant rejection ($P = 0.048$, $P = 0.001$, and $P = 0.010$). Only few studies have evaluated both fragments of M30 and M65-CK 18 in CHB. Zheng et al. found that the M30/M65 CK-18 ratio was statistically significantly higher in the CHB compared to that of the control group and highest in patients with acute chronic liver failure. The combination of these two markers had an AUC of ≥0.80 in the identification of liver failure in patients with CHB, which underlines the markedly marked M65 as a marker for hepatic necrosis [37]. Further work is needed to assess the usefulness of the measurement of CK-18 M56 patients with CHB hepatic failure.

The assessments of liver fibrosis and inflammatory activity are equally important for the clinical evaluation of liver function in chronic HBV infection. Since there are many ultrasound-based and serological noninvasive techniques that allow to evaluate liver fibrosis, there is an obvious need for noninvasive markers of inflammatory activity in the liver, especially in chronic HBV infection. According to EASL guidelines, the active inflammation in the liver in HBeAg(−) patients with HBV-DNA > 2000 U/L is an indication for the antiviral treatment. EASL 2017 Clinical Practice Guidelines allow the use of noninvasive biomarker markers, while do not specify which of them could be used [38]. In our study, we show that serum M30 CK-18 has better accuracy for the evaluation of liver inflammatory activity compared to ALT, while it is simple to use, does not require complex calculation algorithms, and is cheaper compared to commercial ones.

In conclusion, our study showed that serum concentration of M30 CK-18 reflects not only the advancement of fibrosis but also activity of disease. Serum M30 CK-18 > 253U/L has good sensitivity and specificity in discriminating mild versus moderate/severe fibrosis but also active liver inflammation even in patients with normal ALT activity. This study suggests M30 CK-18 as a potential noninvasive marker of CHB activity with clinical advantage compared with ALT activity but also facilitating the recognition the phase of disease.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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References

[1] G. Zacharakis, J. Koskinas, S. Kotsiou et al., "The role of serial measurement of serum HBV DNA levels in patients with chronic HBeAg(−) hepatitis B infection: association with liver disease progression. A prospective cohort study," *Journal of Hepatology*, vol. 49, pp. 884–891, 2008.

[2] G. Fattovich, N. Olivari, M. Pasino, M. D'Onofrio, E. Martone, and F. Donato, "Long-term outcome of chronic hepatitis B in Caucasian patients: mortality after 25 years," *Gut*, vol. 57, pp. 84–90, 2008.

[3] M. R. Brunetto, F. Oliveri, B. Coco et al., "Outcome of anti-HBe positive chronic hepatitis B in alpha-interferon treated and untreated patients: a long term cohort study," *Journal of Hepatology*, vol. 36, pp. 263–270, 2002.

[4] N. Terrault, R. Kim, S. Schalm et al., "Presence of biopsy-proven histologic damage (necroinflammation and fibrosis) is common even when ALT is less than 2xULN in patients with chronic hepatitis B (CHB)," *Journal of Hepatology*, vol. 46, no. S1, p. S184, 2007.

[5] P. Bedossa, D. Dargère, and V. Paradis, "Sampling variability of liver fibrosis in chronic hepatitis C," *Hepatology*, vol. 38, pp. 1449–1457, 2003.

[6] C. T. Wai, J. K. Greenson, R. J. Fontana et al., "A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C," *Hepatology*, vol. 38, pp. 518–526, 2003.

[7] N. H. Afdhal, B. R. Bacon, K. Patel et al., "Accuracy of fibroscan, compared with histology, in analysis of liver fibrosis in patients with hepatitis B or C: a United States multicenter study," *Clinical Gastroenterology and Hepatology*, vol. 13, pp. 772–779, 2015.

[8] N. Sturm, A. Marlu, P. Arvers, J. P. Zarski, and V. Leroy, "Comparative assessment of liver fibrosis by computerized morphometry in naïve patients with chronic hepatitis B and C," *Liver International*, vol. 33, pp. 428–438, 2013.

[9] J. M. Schattenberg, P. R. Galle, and M. Schuchmann, "Apoptosis in liver disease," *Liver International*, vol. 26, pp. 904–911, 2006.

[10] B. D. Maliken, J. E. Nelson, H. M. Klintworth, M. Beauchamp, M. M. Yeh, and K. V. Kowdley, "Hepatic reticuloendothelial system cell iron deposition is associated with increased apoptosis in nonalcoholic fatty liver disease," *Hepatology*, vol. 57, pp. 1806–1813, 2013.

[11] W. Zhang, Z. Guo, L. Zhang et al., "Maternal immunization promotes the immune response of neonates towards hepatitis B vaccine," *Journal of Viral Hepatitis*, vol. 20, pp. 875–881, 2013.

[12] M. Luto, J. Jaroszewicz, and R. Flisiak, "Cytokeratin 18 as an indicator of the activity of liver disease," *Polski Merkuriusz Lekarski*, vol. 31, pp. 331–334, 2011.

[13] H. Bantel, A. Lügering, J. Heidemann et al., "Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury," *Hepatology*, vol. 40, pp. 1078–1087, 2004.

[14] S. Sumer, N. Aktug Demir, S. Kölgelier et al., "The clinical significance of serum apoptotic cytokeratin 18 neoepitope M30 (M30 CK-18) and matrix metalloproteinase 2 (MMP-2) levels in chronic hepatitis B patients with cirrhosis," *Hepatitis Monthly*, vol. 13, no. 6, article e10106, 2013.

[15] D. Micha, J. Cummings, A. Shoemaker et al., "Circulating biomarkers of cell death after treatment with the BH-3 mimetic ABT-737 in a preclinical model of small-cell lung cancer," *Clinical Cancer Research*, vol. 14, pp. 7304–7310, 2008.

[16] L. Petris, E. Brandén, R. Herrmann et al., "Diagnostic and prognostic role of plasma levels of two forms of cytokeratin 18 in patients with non-small-cell lung cancer," *European Journal of Cancer*, vol. 47, pp. 131–137, 2011.

[17] G. V. Papatheodoridis, E. Hadziyannis, E. Tsochatzis et al., "Serum apoptotic caspase activity as a marker of severity in HBeAg-negative chronic hepatitis B virus infection," *Gut*, vol. 57, pp. 500–506, 2008.

[18] G. V. Papatheodoridis, E. Hadziyannis, E. Tsochatzis et al., "Serum apoptotic caspase activity in chronic hepatitis C and nonalcoholic fatty liver disease," *Journal of Clinical Gastroenterology*, vol. 44, pp. 87–97, 2010.

[19] J. Jaroszewicz, B. Calle Serrano, K. Wursthorn et al., "Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV) -infection: a European perspective," *Journal of Hepatology*, vol. 52, pp. 514–522, 2010.

[20] M. R. Brunetto, F. Oliveri, P. Colombatto et al., "Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers," *Gastroenterology*, vol. 139, pp. 483–490, 2010.

[21] M. Martinot-Peignoux, M. Lapalus, T. Asselah, and P. Marcellin, "HBsAg quantification: useful for monitoring natural history and treatment outcome," *Liver International*, vol. 34, no. S1, pp. 97–107, 2014.

[22] M. B. Zeisel, J. Lucifora, W. S. Mason et al., "Towards an HBV cure: state-of-the-art and unresolved questions–report of the ANRS workshop on HBV cure," *Gut*, vol. 64, pp. 1314–1326, 2015.

[23] A. B. Jazwinski, A. J. Thompson, P. J. Clark, S. Naggie, H. L. Tillmann, and K. Patel, "Elevated serum CK-18 levels in chronic hepatitis C patients are associated with advanced fibrosis but not steatosis," *Journal of Viral Hepatitis*, vol. 19, pp. 278–282, 2012.

[24] A. Parfieniuk-Kowerda, T. W. Lapinski, M. Rogalska-Plonska et al., "Serum cytochrome c and m30-neoepitope of cytokeratin-18 in chronic hepatitis C," *Liver International*, vol. 34, pp. 544–550, 2014.

[25] M. Tabuchi, K. Tomioka, T. Kawakami et al., "Serum cytokeratin 18 M30 antigen level and its correlation with nutritional parameters in middle-aged Japanese males with nonalcoholic fatty liver disease (NAFLD)," *Journal of Nutritional Science and Vitaminology*, vol. 56, pp. 271–278, 2010.

[26] H. Bantel, K. John, and K. Schulze-Osthoff, "Robust detection of liver steatosis and staging of NAFLD by an improved ELISA for serum cytokeratin-18 fragments," *The American Journal of Gastroenterology*, vol. 109, pp. 140-141, 2014.

[27] C. B. Bae, S. S. Kim, S. J. Ahn et al., "Caspase-cleaved fragments of cytokeratin-18 as a marker of inflammatory activity in chronic hepatitis B virus infection," *Journal of Clinical Virology*, vol. 58, pp. 641–646, 2013.

[28] Y. Shi, S. Sun, Y. Liu et al., "Keratin 18 phosphorylation as a progression marker of chronic hepatitis B," *Virology Journal*, vol. 7, p. 70, 2010.

[29] H. L. Chan, V. W. Wong, A. M. Tse et al., "Serum hepatitis B surface antigen quantitation can reflect hepatitis B. Virus in the liver and predict treatment response," *Clinical Gastroenterology and Hepatology*, vol. 5, pp. 1462–1468, 2007.

[30] M. Świderska, M. Pawłowska, W. Mazur et al., "Distribution of HBV genotypes in Poland," *Clinical and Experimental Hepatology*, vol. 1, pp. 1–4, 2015.

[31] A. G. Miamen, H. Dong, and L. R. Roberts, "Immunotherapeutic approaches to hepatocellular carcinoma treatment," *Liver Cancer*, vol. 1, no. 3-4, pp. 226–237, 2012.

[32] H. Elalfy, T. Besheer, M. M. Arafa, M. A. El-Hussiny, M. A. El Latif, and S. A. Alsayed, "Caspase-cleaved cytokeratin 18 fragment M30 as a potential biomarker of macrovascular invasion in hepatocellular carcinoma," *Journal of Gastrointestinal Cancer*, 2017.

[33] Y. Ngo, Y. Benhamou, V. Thibault et al., "An accurate definition of the status of inactive hepatitis B virus carrier by a combination of biomarkers (FibroTest-ActiTest) and viral load," *PLoS One*, vol. 3, no. 7, article e2573, 2008.

[34] L. A. Adams, M. Bulsara, E. Rossi et al., "Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection," *Clinical Chemistry*, vol. 51, no. 10, pp. 1867–1873, 2005.

[35] D. Joka, K. Wahl, S. Moeller et al., "Prospective biopsy-controlled evaluation of cell death biomarkers for prediction of liver fibrosis and nonalcoholic steatohepatitis," *Hepatology*, vol. 55, pp. 455–464, 2012.

[36] H. Reis, J. Wohlschläger, S. Hagemann et al., "(Cleaved) CK-18 serum and tissue expression levels differentiate acute HCV reinfection from acute rejection in liver allografts," *Liver International*, vol. 37, no. 3, pp. 905–913, 2015.

[37] S. J. Zheng, S. Liu, M. Liu et al., "Prognostic value of M30/M65 for outcome of hepatitis B virus-related acute-on-chronic liver failure," *World Journal of Gastroenterology*, vol. 20, no. 9, pp. 2403–2411, 2014.

[38] "EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection," *Journal of Hepatology*, 2017.