﻿﻿﻿**Materials and Methods**

﻿﻿***RNA library construction and sequencing.*** Total RNA was isolated and purified from liver tissues of 4 healthy controls, 5 patients with CHB, 5 patients with LF/LC and 5 patients with HCC using Trizol DP431 reagent (Tiangen, Beijing, China) following the manufacturer's procedure. Each sample’s RNA amount and purity was quantified using NanoDrop ND-1000 (NanoDrop, Wilmington, DE, USA). The RNA integrity was assessed by Agilent 2100 with RIN number >7.0. Approximately 5 ug of total RNA was used to deplete ribosomal RNA, according to the manuscript of the Ribo-Zero Gold rRNA Removal Kit (Illumina, San Diego, USA). After removing ribosomal RNAs, the left RNAs were fragmented into small pieces using divalent cations under high temperature. Then the cleaved RNA fragments were reverse-transcribed to create the cDNA, which were next used to synthesise U-labeled second-stranded DNAs with E. coli DNA polymerase I, RNase H and dUTP. An A-base is then added to the blunt ends of each strand, preparing them for ligation to the indexed adapters. Each adapter contains a T-base overhang for ligating the adapter to the A-tailed fragmented DNA. Single-or dual-index adapters are ligated to the fragments, and size selection was performed with AMPureXP beads. After the heat-labile UDG enzyme treatment of the U-labeled second-stranded DNAs, the ligated products are amplified with PCR by the following conditions: initial denaturation at 95℃ for 3 min; 8cycles of denaturation at 98℃ for 15 sec, annealing at 60℃ for 15 sec, and extension at 72℃ for 30 sec; and then final extension at 72℃ for 5 min. The average insert size for the final cDNA library was 300 bp (±50 bp). At last, the paired-end sequencing was performed on an Illumina NovaSeqTM 6000 (LC Bio, China) following the vendor's recommended protocol.

﻿﻿﻿***Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis.*** ﻿Total RNA was isolated from liver tissues and PBMCs by TRIzol reagent (Invitrogen). First-strand cDNA was synthesized using a PrimeScript RT Reagent Kit (Takara, Japan) according to instructions suggested by the manufacturer. Quantitative PCR was performed in ABI 7500 real-time PCR system (Applied Biosystems, Carlsbad, CA) using TB Green™ Premix Ex Taq™ II (Tli RNaseH Plus) (Takara, Japan). Primer sequences are listed in Table 1. The reaction mixtures were pre-degenerated at 95 C for 30 s, followed by 40 cycles of 95 C for 10 s and 60 C for 30 s. GAPDH expression was included as an internal control. The 2−ΔΔCt method was used to calculate the relative expression of RNAs. Each assay was performed in triplicates and repeated three times.

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**Table S1.** ﻿Inclusion and Exclusion Criteria

|  |
| --- |
| **Inclusion Criteria** |
| ﻿Age, ≥ 18 years |
| Meeting criteria for the diagnosis of each disease |
| Individuals who don’t take Chinese medicines and health products a year recently |
| Individuals who agree to sign written informed consents |
| **Exclusion Criteria** |
| Coinfection with hepatitis C virus, hepatitis D virus, human immunodeficiency virus and autoimmune, genetic or metabolic liver diseases |
| Pregnancy |
| Severe alcohol abuse |
| ﻿Serious medical comorbidities (severe cardiovascular diseases, diabetes, etc.) |
| Hematologic disorders |
| Coexistence of malignant tumors |

**﻿Table S2.** Clinical and laboratory characteristics of the participants in the test set

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **Healthy control**  **(n = 100)** | **Chronic Hepatitis B**  **(n = 100)** | **Liver fibrosis/cirrhosis**  **(n = 100)** | **Hepatocellular carcinoma**  **(n = 100)** | **F/Z/X2** | ***P*** |
| Gender (male), n (%) | 49 (49.0) | 64 (64.0)\* | 75 (75.0)\* | 80 (80.0)\*# | 25.599 | 0.000 |
| Age (years) | 31.0 (24.0-39.0) | 40.0 (31.0-52.0)\* | 47.0 (38.0-56.0)\*# | 57.0 (50.0-64.0)\*#& | 152.931 | 0.000 |
| WBC (109/L) | 6.0 (5.4-6.9) | 5.1 (4.3-6.2)\* | 4.8 (3.5-6.5)\* | 4.6 (2.7-5.8)\*# | 49.027 | 0.000 |
| HGB (g/L) | 142.8 (132.7-154.3) | 142.5 (128.7-156.8) | 136.4 (118.3-152.7)\*# | 129.0 (109.0-140.0)\*#& | 39.168 | 0.000 |
| PLT (109/L) | 246.2 (211.4-277.5) | 191.0 (161.0-230.0)\* | 110.0 (68.3-154.5)\*# | 99.5 (60.3-127.4)\*#& | 220.780 | 0.000 |
| ALB (g/L) | 49.1 (47.1-50.7) | 44.4 (40.6-47.7)\* | 41.8 (37.8-45.6)\*# | 35.7 (30.7-41.9)\*#& | 164.179 | 0.000 |
| ALT (U/L) | 15.0 (10.0-22.8) | 30.5 (18.0-52.3)\* | 34.0 (22.0-51.8)\* | 34.5 (22.0-52.8)\* | 93.211 | 0.000 |
| AST (U/L) | 17.0 (14.3-20.8) | 25.5 (17.0-41.8)\* | 29.0 (21.0-47.8)\*# | 37.0 (25.3-63.3)\*#& | 128.710 | 0.000 |
| TBIL (μmol/L） | 14.1 (11.8-17.0) | 16.1 (11.8-19.6) | 20.8 (14.2-34.0)\*# | 26.5 (17.6-52.8)\*#& | 82.070 | 0.000 |
| CREA (μmol/L) | 66.7 (56.6-78.9) | 62.6 (53.0-72.7)\* | 62.3 (51.7-68.9)\* | 58.3 (51.5-72.5)\* | 10.557 | 0.014 |
| PT (s) | — | 11.1 (10.7-12.3) | 13.0 (11.8-14.3)# | 12.8 (11.4-14.5)# | 43.655 | 0.000 |
| INR | — | 1.00 (0.94-1.09) | 1.16 (1.05-1.29)# | 1.13 (1.01-1.30)# | 44.270 | 0.000 |
| HBV-DNA  (log10 IU/mL) | — | 5.0 (2.2-7.3) | 1.8 (1.3-5.3)# | 2.2 (1.3-3.4)# | 38.276 | 0.000 |
| HBsAg (log10 IU/mL) | — | 3.4 (3.0-3.9) | 2.9 (2.4-3.4)# | 2.4 (2.3-2.7)#& | 76.522 | 0.000 |
| AFP (ng/mL) | 3.6 (2.5-4.8) | 3.6 (2.2-5.8) | 4.4 (2.4-19.8)\*# | 10.6 (3.6-699.9)\*#& | 60.439 | 0.000 |
| Ferritin (ng/mL) | 35.0 (28.1-62.2) | 81.3 (39.3-142.6)\* | 130.8 (62.4-231.8)\*# | 224.4 (138.0-545.1)\*#& | 129.819 | 0.000 |
| FIB-4 | 0.58 (0.45-0.78) | 0.92 (0.67-1.49)\* | 2.24 (1.47-4.28)\*# | 5.42 (2.68-7.94)\*#& | 244.942 | 0.000 |
| APRI | 0.2 (0.1-0.2) | 0.3 (0.2-0.5)\* | 0.8 (0.4-1.3)\*# | 1.4 (0.6-2.3)\*#& | 233.815 | 0.000 |
| LSM (kPa) | — | 7.4 (5.9-10.2) | 14.9 (8.7-18.8)# | — | 42.985 | 0.000 |
| SHC1 (ng/mL) | 5.8 (4.8-7.2) | 7.9 (6.4-9.1)\* | 9.6 (8.0-10.8)\*# | 11.4 (9.8-12.5)\*#& | 228.763 | 0.000 |
| SLAMF8 (ng/mL) | 4.6 (3.8-5.2) | 6.1 (5.3-6.8)\* | 7.5 (6.7-8.4)\*# | 8.8 (7.8-9.6)\*#& | 302.448 | 0.000 |
| IL-32 (pg/mL) | 34.9 (27.4-41.8) | 49.4 (42.2-59.5)\* | 68.2 (58.3-76.3)\*# | 84.8 (77.1-92.7)\*#& | 316.031 | 0.000 |

**﻿Notes:** \*was defined as *P* < 0.05 vs healthy control. #wasdefined as *P* < 0.05 vs chronic hepatitis B. &was defined as *P* < 0.05 vs liver fibrosis/cirrhosis.

**Abbreviations:** WBC: white blood cell; HGB: hemoglobin; PLT: platelet; ALB: albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; CREA: creatinine; PT: prothrombin time; INR: international normalized ratio; HBsAg: hepatitis B surface antigen; AFP: alpha fetoprotein; FIB-4: Fibrosis index based on the 4 factor; APRI: AST-to-PLT ratio index; LSM: liver stiffness measurement; SHC1: SHC adaptor protein 1; SLAMF8: SLAM family member 8; IL-32: interleukin-32.

**Table S3.** Comparison of diagnostic performance for plasma SHC1, SLAMF8, IL-32 ﻿and new model for patients with different stages of disease in the test set

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Variables | AUC | Cut-off | Sensitivity | Specificity | Youden index |
| Chronic hepatitis B | SHC1 (ng/mL) | 0.791 | 7.719 | 57.0 | 88.0 | 0.450 |
| SLAMF8 (ng/mL) | 0.911 | 4.938 | 99.0 | 66.0 | 0.650 |
| IL-32 (pg/mL) | 0.863 | 42.736 | 75.0 | 82.0 | 0.570 |
| APFSSI | 0.980 | 1.683 | 97.0 | 88.0 | 0.850 |
| Liver fibrosis/cirrhosis | SHC1 (ng/mL) | 0.730 | 9.742 | 49.0 | 87.0 | 0.360 |
| SLAMF8 (ng/mL) | 0.841 | 6.945 | 68.0 | 86.0 | 0.540 |
| IL-32 (pg/mL) | 0.883 | 64.842 | 59.0 | 99.0 | 0.580 |
| APFSSI | 0.958 | 2.524 | 94.0 | 90.0 | 0.840 |
| Hepatocellular carcinoma | SHC1 (ng/mL) | 0.744 | 11.372 | 52.0 | 87.0 | 0.390 |
| SLAMF8 (ng/mL) | 0.791 | 8.685 | 56.0 | 91.0 | 0.470 |
| IL-32 (pg/mL) | 0.852 | 75.934 | 83.0 | 75.0 | 0.580 |
| APFSSI | 0.930 | 3.469 | 88.0 | 84.0 | 0.720 |

**Abbreviations:** AUC: area under the curve; SHC1: SHC adaptor protein 1; SLAMF8: SLAM family member 8; IL-32: interleukin-32.

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**Figure S1.** Comparative transcriptomics analysis of CON, CHB, LF/LC and HCC.(A) Differentially expressed mRNAs in different groups. (B) Venn diagram showing the differential expression of mRNAs. Volcano plots for differentially expressed mRNAs between CHB and CON (C), LF/LC and CON (D) and HCC and CON (E).

﻿**Abbreviations:** CHB: chronic hepatitis B; CON: healthy control; HCC: hepatocellular carcinoma; LF: liver fibrosis; LC: liver cirrhosis.



**Figure S2.** Further evaluation of the APFSSI model.Plasma (A) SHC1, (B) SLAMF8 and (C) IL-32 levels in the four groups. (D) ROC curves of all 3 diagnostic biomarkers and the APFSSI model to differentiate CHB patients from healthy subjects in the test sample set. (E) Comparisons of the sensitivity and specificity of the mRNA panel and other variables in the test set for LF/LC diagnosis prediction. (F) ROC curves for measuring the performance of mRNA biomarkers to distinguish HCC patients from LF/LC patients. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001.

**Abbreviations:** CHB: chronic hepatitis B; CON: healthy control; HCC: hepatocellular carcinoma; IL-32: interleukin-32; LF: liver fibrosis; LC: liver cirrhosis; SHC1: SHC adaptor protein 1; SLAMF8: SLAM family member 8; ROC: receiver operating characteristic.