

ADDITIONAL FILE 1

Thrombospondin 1 enhances systemic inflammation and disease severity in acute-on-chronic liver failure

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Table S1. Clinical characteristics of all enrolled subjects

Characteristics	ACLF (n=130)	LC (n=70)	CHB (n=70)	NC (n=60)
Age (yrs.)	44.5 [36.2, 55.0]	46.0 [40.0, 52.5]	43.5 [38.0, 50.0]	46.5 [33.8, 53.0]
Male (%)	86.2% (112)	74.3% (52)	77.1% (54)	56.7% (34) ***
HBV DNA level (IU/mL)		Significance [†]	Significance [†]	
≤ 2×10 ²	5.4% (7)	91.4% (64)	81.4% (57)	NA
2×10 ² – 2×10 ⁶	66.9% (87)	8.6% (6)	12.9% (9)	NA
> 2×10 ⁶	27.7% (36)	0.0% (0)	5.7% (4)	NA
Laboratory data				
Alanine aminotransferase (U/L)	252.0 [93.0, 592.0]	24.5 [18.0, 32.0] ***	23.2 [17.0, 29.8] ***	19.0 [14.0, 25.0] ***
Aspartate aminotransferase (U/L)	147.5 [101.3, 290.8]	23.5 [19.3, 29.8] ***	22.0 [20.0, 26.8] ***	20.0 [18.0, 25.0] ***
Albumin (g/dL)	31.5 [28.5, 33.8]	47.6 [45.6, 48.8] ***	48.7 [46.5, 51.1] ***	47.7 [46.1, 49.1] ***
Total bilirubin (μmol/L)	352.5 [262.9, 437.9]	13.2 [11.0, 17.0] ***	13.0 [9.2, 16.0] ***	11.0 [8.5, 14.0] ***
Alkaline phosphatase (U/L)	137.0 [110.5, 166.0]	70.0 [58.3, 90.0] ***	64.5 [50.5, 79.8] ***	67.00 [55.0, 84.0] ***
γ-Glutamyl transpeptidase (U/L)	77.8 [56.0, 115.4]	22.0 [17.0, 37.8] ***	20.5 [13.0, 32.8] ***	19.0 [12.8, 27.0] ***
Creatinine (μmol/L)	67.0 [58.0, 80.8]	70.5 [62.7, 81.0]	75.0 [65.0, 85.0]	70.5 [57.7, 81.0]
Sodium (mmol/L)	137.0 [135.0, 139.0]	141.0 [140.0, 142.0] ***	141.0 [140.0, 143.0] ***	141.0 [140.0, 143.0] ***
White blood cell count (10 ⁹ /L)	7.6 [6.1, 10.3]	5.1 [4.0, 6.3] ***	5.5 [4.7, 6.3] ***	6.1 [5.4, 6.9] ***

Characteristics	ACLF (n=130)	LC (n=70)	CHB (n=70)	NC (n=60)
Hemoglobin (g/L)	125.0 [116.2, 136.0]	153.0 [142.5, 158.8] ***	151.0 [140.0, 162.0] ***	149.0 [136.8, 160.0] ***
Hematocrit (%)	35.6 [32.5, 38.9]	45.5 [42.9, 47.3] ***	44.5 [41.2, 46.9] ***	44.1 [40.7, 47.6] ***
Platelet count (10 ⁹ /L)	112.0 [74.0, 145.0]	155.5 [117.0, 207.8] ***	178.9 ± 63.3 ***	218.5 [188.5, 257.2] ***
INR	2.3 [1.8, 3.0]	1.0 [1.0, 1.1] ***	0.97 [0.94, 1.02] ***	NA
Alpha fetoprotein	88.8 [34.8, 235.3]	2.2 [1.8, 2.8] ***	2.5 [1.9, 3.4] ***	2.8 [2.0, 4.0] ***
Organ failure (%)				
Liver	94.6% (123)	NA	NA	NA
Coagulation	42.3% (55)	NA	NA	NA
Kidneys	6.2% (8)	NA	NA	NA
Brain	7.7% (10)	NA	NA	NA
Lungs	1.5% (2)	NA	NA	NA
Circulation	0.8% (1)	NA	NA	NA
Severity score				
COSSH-ACLF IIs	7.3 [6.7, 7.8]	NA	NA	NA
COSSH-ACLFs	6.2 [5.7, 6.8]	NA	NA	NA

Characteristics	ACLF (n=130)	LC (n=70)	CHB (n=70)	NC (n=60)
CLIF-C ACLFs	42.0 [37.3, 47.1]	NA	NA	NA
MELDs	24.6 [20.5, 28.3]	NA	NA	NA
MELD-Nas	25.7 [22.0, 29.5]	NA	NA	NA
CTP	10.0 [9.0, 11.0]	5.0 [5.0, 5.0]	5.0 [5.0, 5.0]	NA
Transplant-free mortality rate (number of deceased patients) ‡				
28-day	33.0% (37)	NA	NA	NA
90-day	46.0% (47)	NA	NA	NA

NOTE. Data are presented as the means \pm SDs, medians with [p25, p75], or percentages (numbers of patients). ACLF, acute-on-chronic liver failure; LC, liver cirrhosis; CHB, chronic hepatitis B; NC, normal control; COSSH-ACLFs, COSSH-ACLF score; CLIF-C ACLFs, CLIF Consortium ACLF score; MELDs, Model for End-stage Liver Disease score; MELD-Nas, MELD-sodium score; CTP, Child-Turcotte-Pugh score.

‡Twelve patients with ACLF underwent liver transplantation and were considered lost to follow-up in the mortality rate calculation. Six patients with ACLF were lost to the 28-day follow-up analysis, and 16 patients were lost to the 90-day follow-up analysis.

† *P* value (<0.001) for comparisons of the HBV DNA distribution between the LC/CHB/NC and ACLF groups.

*** *P*<0.001 for comparisons between the LC/CHB/NC and ACLF groups.

Table S2. Clinical characteristics of ACLF patients in the ELISA validation group

Characteristics	All HBV-ACLF (n = 198)	Non-Survivor[†] (n = 91)	Survivor[†] (n = 107)
Age (yrs.)	48.3±11.9	52.7±11.3	44.6±11.2
Male (%)	81.3% (161)	80.2% (73)	82.2% (88)
HBV DNA level (IU/mL)			
≤ 2×10 ²	5.6% (11)	4.4% (4)	8.4% (9)
2×10 ² – 2×10 ⁶	65.6% (130)	62.6% (57)	66.4% (71)
> 2×10 ⁶	28.8% (57)	33.0% (30)	25.2% (27)
Laboratory data			
Alanine aminotransferase (U/L)	224.0 [90.0, 443.0]	218.0 [91.0, 476.0]	244.0 [82.0, 435.0]
Aspartate aminotransferase (U/L)	130.5 [78.8, 277.5]	147.0 [86.0, 323.0]	130.0 [75.0, 216.0]
Albumin (g/dL)	30.7 [28.2, 33.0]	30.0 [27.7, 32.6]	31.1 [28.6, 34.1] *
Total bilirubin (μmol/L)	343.6 [277.7, 427.6]	398.4 [307.5, 452.3]	306.4 [253.5, 378.0] ***
Alkaline phosphatase (U/L)	143.5 [120.0, 176.3]	142.0 [118.0, 179.0]	146.0 [121.0, 176.0]
γ-Glutamyl transpeptidase (U/L)	76.0 [49.0, 120.5]	68.0 [48.0, 114.0]	77.0 [50.0, 123.0]
Creatinine (μmol/L)	61.5 [54.0, 76.3]	63.0 [54.0, 85.0]	60.0 [53.0, 71.0] **

Characteristics	All HBV-ACLF (n = 198)	Non-Survivor[†] (n = 91)	Survivor[†] (n = 107)
Serum urea (mmol/L)	4.3 [3.2, 6.2]	5.1 [3.6, 7.6]	3.9 [2.9, 5.2] ***
Triglycerides (mmol/L)	1.2 [0.9, 1.5]	1.0 [0.8, 1.3]	1.3 [1.0, 1.8] ***
Total cholesterol (mmol/L)	2.0 [1.6, 2.6]	1.8 [1.5, 2.3]	2.2 [1.8, 2.7] **
Sodium (mmol/L)	138.0 [136.0, 140.0]	137.0 [135.0, 139.0]	138.0 [136.0, 140.0]
White blood cell count (10 ⁹ /L)	7.5 [5.9, 9.6]	7.8 [6.3, 10.3]	6.9 [5.0, 9.4] **
Neutrophils (10 ⁹ /L)	5.3 [3.6, 7.1]	5.7 [4.3, 8.2]	4.5 [3.1, 6.8] ***
Hemoglobin (g/L)	122.0 [107.0, 137.0]	121.0 [107.0, 136.0]	123.0 [108, 138.0]
Hematocrit (%)	33.8 [29.9, 39.1]	33.2 [29.7, 38.1]	34.1 [30.5, 39.5]
Platelet count (10 ⁹ /L)	100.5 [69.0, 136.3]	91.0 [69.0, 124.0]	110.0 [70.0, 149] *
INR	2.1 [1.8, 2.7]	2.5 [2.0, 3.2]	1.9 [1.6, 2.3] ***
Fibrinogen (g/L)	1.3 [1.0, 1.6]	1.2 [0.9, 1.5]	1.4 [1.1, 1.7] **
Prothrombin time (s)	23.6 [19.9, 29.1]	28.7 [22.7, 34.0]	21.0 [18.6, 25.2] ***
D-dimer (μg/L)	2315.0 [1098.0, 4228.0]	3496.0 [1840.0, 5031.0]	1742.0 [751.0, 3290.0] ***
Alpha fetoprotein	93.5 [30.0, 261.3]	88.6 [23.8, 222.7]	116.7 [30.1, 294.6]
Ferritin (μg/L)	2664.0 [1689.0, 4789.0]	3333.0 [2050.0, 5922.0]	2347.0 [1286.0, 4024.0] **

Characteristics	All HBV-ACLF (n = 198)	Non-Survivor[†] (n = 91)	Survivor[†] (n = 107)
Organ failure (%)			
Liver	98.5% (195)	97.8% (89)	99.1% (106)
Coagulation	34.9% (69)	55.0% (50)	17.8% (19) ***
Kidneys	4.0% (8)	8.8% (8)	0.0% (0) ***
Brain	9.6% (19)	17.6% (16)	2.8% (3) ***
Lungs	1.5% (3)	3.3% (3)	0.0% (0)
Circulation	3.0% (6)	5.5% (5)	0.9% (1)
Severity score			
COSSH-ACLFs	6.2 [5.7, 6.9]	6.8 [6.3, 7.6]	5.8 [5.4, 6.2] ***
COSSH-ACLF IIs	7.2 [6.6, 8.0]	8.0 [7.3, 8.5]	6.7 [6.3, 7.1] ***
CLIF-C ACLFs	42.5 [37.4, 48.2]	47.7 [43.1, 51.3]	38.7 [34.3, 42.4] ***
MELDs	26.6 [24.3, 29.3]	29.0 [26.6, 32.7]	25.0 [23.2, 27.0] ***
MELD-Nas	27.2 [25.2, 30.1]	29.6 [27.1, 32.9]	25.8 [23.9, 27.3] ***
CTP	11.0 [10.0, 12.0]	12.0 [11.0, 12.0]	11.0 [10.0, 11.0] ***

Characteristics	All HBV-ACLF (n = 198)	Non-Survivor [†] (n = 91)	Survivor [†] (n = 107)
ACLF grade			
ACLF-1	62.6% (124)	40.7% (37)	81.3% (87) **
ACLF-2	28.3% (56)	40.7% (37)	17.8% (19) **
ACLF-3	9.1% (18)	18.6% (17)	0.9% (1) ***
Transplant-free mortality rate (number of deceased patients)			
28-day	33.3% (66)	72.5% (66)	0.0% (0) ***
90-day	46.0% (91)	100.0% (91)	0.0% (0) ***

NOTE. Data are presented as the means ± SDs, medians with [p25, p75], or percentages (numbers of patients). ACLF, acute-on-chronic liver failure; COSSH-ACLFs, Chinese Group on the Study of Severe Hepatitis B-ACLF score; COSSH-ACLF IIs, COSSH-ACLF II score; CLIF-C ACLFs, Chronic Liver Failure- Consortium ACLF score; MELDs, model for end-stage liver disease score; MELD-Nas, MELD-sodium score; CTP, child-turcotte-pugh score.

[†] Non-survivor, HBV-ACLF patients who died within 90 days; Survivor, HBV-ACLF patients who survived 90 days.

P* value (<0.05), *P* value (<0.01) and ****P* value (<0.001) for comparisons between non-survivor and survivor group.

Table S3. Primers used for real-time PCR

Gene	Primer orientation	Nucleotide sequence	NCBI Gene ID number
<i>hGAPDH</i>	forward	5'-CTCTCTGCTCCTCCTGTTCG-3'	2597
	reverse	5'-ACGACCAAATCCGTTGACTC-3'	
<i>hTHBS1</i>	forward	5'-AGGCATGTTCCAGTTTCACC-3'	7057
	reverse	5'-GCTGGCACCACCTTTATTGT-3'	
<i>rGAPDH</i>	forward	5'-ATGGAGAAGGCTGGGGCTCACCT-3'	24383
	reverse	5'-AGCCCTTCCACGATGCCAAAGTTGT-3'	
<i>rTHBS1</i>	forward	5'-TAGCTGGAAATGTGGTGCGT-3'	445442
	reverse	5'-TCAGCTTCACATTGCCTGTC-3'	
<i>rCCL2</i>	forward	5'-ATGCAGTTAATGCCCCACTC-3'	24770
	reverse	5'-TTCCTTATTGGGGTCAGCAC-3'	
<i>rIL-10</i>	forward	5'- CCTGCTCTTACTGGCTGGAG -3'	25325
	reverse	5'-TGTCCAGCTGGTCCTTCTTT -3'	
<i>rIL-6</i>	forward	5'-AGAGACTTCCAGCCAGTTGC-3'	24498
	reverse	5'-AGTCTCCTCTCCGGACTTGT-3'	
<i>rIL-1β</i>	forward	5'-CCTGTTCTTTGAGGCTGACA-3'	24494
	reverse	5'-GCTGTGAGATTTGAAGCTGGA-3'	
<i>rINFγ</i>	forward	5'-ACAACCCACAGATCCAGCAC-3'	25712
	reverse	5'-CCAGAATCAGCACCGACTCC-3'	
<i>rTNFAIP6</i>	forward	5'-AAGCAGCCAGAAAGATTGGA -3'	84397
	reverse	5'-TTCGGGTTGTAGCAATAGGC -3'	
<i>mGAPDH</i>	forward	5'-CATCACTGCCACCCAGAAGACTG-3'	21825
	reverse	5'-ATGCCAGTGAGCTTCCCGTTCAG-3'	
<i>mTHBS1</i>	forward	5'-GGTAGCTGGAAATGTGGTGCGT-3'	14433
	reverse	5'-GCACCGATGTTCTCCGTTGTGA-3'	
<i>mCcl2</i>	forward	5'-GCTACAAGAGGATCACCAGCAG-3'	20296
	reverse	5'-GTCTGGACCCATTCCTTCTTGG-3'	

Gene	Primer orientation	Nucleotide sequence	NCBI Gene ID number
<i>mIL-6</i>	forward	5'-CTGCAAGAGACTTCCATCCAG-3'	16193
	reverse	5'-AGTGGTATAGACAGGTCTGTTGG-3'	
<i>mIL-1β</i>	forward	5'-TGGACCTTCCAGGATGAGGACA-3'	16176
	reverse	5'-GTTTCATCTCGGAGCCTGTAGTG-3'	
<i>mINFγ</i>	forward	5'-CAGCAACAGCAAGGCGAAAAAGG-3'	15978
	reverse	5'-TTTCCGCTTCCTGAGGCTGGAT-3'	
<i>mTNFAIP6</i>	forward	5'-GGATGCCTATTGCTACAACCCAC-3'	21930
	reverse	5'-CTTGAGCCGAATGTGCCAGTAG-3'	
<i>mTNFα</i>	forward	5'-GGTGCCTATGTCTCAGCCTCTT-3'	21926
	reverse	5'-GCCATAGAACTGATGAGAGGGAG-3'	
<i>mIL-10</i>	forward	5'-CGGGAAGACAATAACTGCACCC-3'	16153
	reverse	5'-CGGTTAGCAGTATGTTGTCCAGC-3'	
<i>mIL-1Rn</i>	forward	5'-TGTGCCTGTCTTGTGCCAAGTC-3'	16181
	reverse	5'-GCCTTTCTCAGAGCGGATGAAG-3'	
<i>mIL-4</i>	forward	5'-ATCATCGGCATTTTGAACGAGGTC-3'	16189
	reverse	5'-ACCTTGGAAGCCCTACAGACGA-3'	
<i>mIL-11</i>	forward	5'-CTGACGGAGATCACAGTCTGGA-3'	16156
	reverse	5'-GGACATCAAGTCTACTCGAAGCC-3'	
<i>mIL-13</i>	forward	5'-AACGGCAGCATGGTATGGAGTG-3'	6163
	reverse	5'-TGGGTCCTGTAGATGGCATTGC-3'	

NOTE. *h*, human; *m*, mouse; *r*, rat.

Table S4. Antibodies for Western immunoblotting and immunohistochemistry

Primary antibodies		
Antibody	Catalog number	Company
<i>THBS1</i>	ab1823	Abcam, Cambridge, UK
<i>ALB</i>	HPA031025	Sigma–Aldrich, MO, USA
<i>CD86</i>	ab53004	Abcam, Cambridge, UK
<i>BAX</i>	14796	Cell Signaling Technology (CST), MA, USA
<i>Bcl2</i>	sc-7382	Santa Cruz, CA, USA
<i>Cleaved caspase 3</i>	9661	Cell Signaling Technology (CST) MA, USA
<i>β-actin</i>	Ab8226	Abcam, Cambridge, UK
Secondary antibodies		
Antibody	Catalog number	Company
<i>Goat anti-mouse</i>	ab6789	Abcam, Cambridge, UK
<i>Goat anti-rabbit</i>	ab6721	Abcam, Cambridge, UK

Table S5. Clinical characteristics of ACLF patients with different THBS1 expression in the validation group

Characteristics	Group 0	Group 1
	THBS1<median (3.5) n = 49	THBS1>median (3.5) n = 49
Age (yrs.)	45.5±10.6	47.7±13.6
Male (No.)	85.7% (42)	92.8% (45)
HBV-DNA level (IU/ml)		
$2 \times 10^2 - 2 \times 10^4$	30.6% (15)	22.5% (11)
$2 \times 10^4 - 2 \times 10^6$	40.8% (20)	36.7% (18)
$> 2 \times 10^6$	28.6% (14)	40.8% (20)
Laboratory data		
Alanine aminotransferase (U/L)	225.0 [87.0, 546.0]	368.0 [178.0, 773.0]
Aspartate aminotransferase (U/L)	148.0 [84.0, 290.0]	187.0 [117.0, 368.0]
Albumin (g/dL)	32.0 [28.0, 35.0]	31.5 [28.5, 33.2]
Total bilirubin ($\mu\text{mol/l}$)	342.0 [245.0, 432.0]	344.0 [261.0, 405.3]
Alkaline phosphatase (U/L)	133.0 [100.0, 162.0]	137.0 [112.0, 162.0]
γ -Glutamyl transpeptidase (U/L)	67.0 [38.0, 108.0]	76.0 [47.0, 117.0]**
Creatinine ($\mu\text{mol/L}$)	63.0 [55.0, 72.0]	69.0 [59.0, 91.0]**
Sodium (mmol/L)	137.0 [135.0, 139.0]	137.0 [135.0, 141.0]
White blood cell count ($10^9/\text{L}$)	7.6 [6.1, 9.7]	7.50 [6.2, 10.4]
Hemoglobin (g/L)	126.0 [117.0, 136.0]	125.0 [117.0, 140.0]
Hematocrit (%)	36.6 [33.2, 38.7]	35.6 [33.0, 39.70]
Platelet count ($10^9/\text{L}$)	106.0 [80.0, 172.0]	116.0 [72.0, 133.0]
INR	2.1 [1.7, 2.7]	2.4 [1.8, 2.9]
Alpha fetoprotein	92.4 [33.1, 231.5]	86.4 [30.6 235.3]
Organ failure (No.)		
Liver	91.8% (45)	93.9% (46)

Characteristics	Group 0	Group 1
	THBS1<median (3.5)	THBS1>median (3.5)
	n = 49	n = 49
Coagulation	30.6% (15)	49.0% (24)
Kidneys	0.0% (0)	4.1% (2)
Brain	4.1% (2)	8.2% (4)
Lungs	0.0% (0)	2.0% (1)
Circulation	0.0% (0)	2.0% (1)
ACLF grade		
ACLF-1	73.5% (36)	51.0% (25)
ACLF-2	22.4% (11)	40.8% (20)
ACLF-3	4.1% (2)	8.2% (4)
Severity score		
COSSH-ACLF IIs	7.1 [6.6, 7.5]	7.3 [6.7, 8.1]*
COSSH-ACLFs	6.0 [5.6, 6.7]	6.3 [5.8, 6.9]
CLIF-C ACLFs	40.5 [37.3, 45.1]	41.7 [38.7, 47.2]
MELD-Na	25.4 [20.7, 26.9]	25.9 [22.6, 31.7]
MELD	22.5 [20.2, 26.1]	25.5 [21.6, 30.8]*
CTP	9.0 [8.0, 10.0]	10.0 [9.0, 11.0]*
Transplant-free mortality (No. deceased patients) †		
28-day	17.5% (7)	45.0% (18)*
90-day	27.7% (10)	58.3% (21)*

NOTE. Data are expressed as the mean ± standard deviation (SD), median (p25, p75) or percentage (number of patients).

COSSH-ACLF IIs, COSSH-ACLF II score; COSSH-ACLFs, COSSH-ACLF score; CLIF-C ACLFs, CLIF Consortium ACLF score; MELDs, Model for End-stage Liver Disease score; MELD-Na, MELD-sodium score; CTP, Child-Turcotte-Pugh score; THBS1, thrombospondin 1.

†Twelve patients with ACLF underwent liver transplantation and were considered lost to follow-up in the mortality rate calculation. Six patients with ACLF were lost to the 28-day follow-up analysis, and 14 patients were lost to the 90-day follow-up analysis.

* $P < 0.05$ and ** $P < 0.01$ for comparisons between the groups.

Table S6. Clinical characteristics of ACLF patients in the low-risk group and high-risk group of the ELISA validation cohort

Characteristics	Low-risk group n = 75	High-risk group n = 123
Age (yrs.)	50.3±12.0	48.2±12.2
Male (No.)	78.7% (59)	82.9% (102)
HBV-DNA level (IU/ml)		
$2 \times 10^2 - 2 \times 10^4$	37.3% (28)	36.6% (45)
$2 \times 10^4 - 2 \times 10^6$	44.0% (33)	34.1% (42)
$> 2 \times 10^6$	18.7% (14)	29.3% (36)
Laboratory data		
Alanine aminotransferase (U/L)	152.0 [55.0, 318.0]	250.0 [100.0, 476.0]*
Aspartate aminotransferase (U/L)	114.0 [67.0, 211.0]	130.0 [80.0, 294.0]
Albumin (g/dL)	30.6 [27.9, 33.8]	30.5 [28.3, 33.2]
Total bilirubin ($\mu\text{mol/l}$)	310.0 [252.1, 416.5]	366.1 [282.0, 428.9]
Alkaline phosphatase (U/L)	129.0 [109.0, 173.0]	143.0 [122.0, 174.0]
γ -Glutamyl transpeptidase (U/L)	68.0 [39.0, 109.0]	79.0 [54.0, 122.0]*
Creatinine ($\mu\text{mol/L}$)	65.0 [58.0, 80.0]	60.0 [52.0, 75.0]
Sodium (mmol/L)	138.0 [135.0, 140.0]	138.0 [136.0, 140.0]
White blood cell count ($10^9/\text{L}$)	6.50 [4.5, 9.1]	7.7 [6.1, 9.6]
Hemoglobin (g/L)	112.0 [103.0, 133.0]	125.0 [110.0, 139.0]*
Hematocrit (%)	31.5 [29.6, 38.1]	34.6 [30.8, 39.1]
Platelet count ($10^9/\text{L}$)	76 [55.0, 117.0]	113.0 [78.0, 141.0]***
INR	2.24 [1.79, 2.67]	2.10 [1.80, 2.64]
Alpha fetoprotein	104.1 [29.1, 309.8]	90.5 [33.7, 257.2]
Organ failure (No.)		
Liver	96.0% (72)	100.0% (123)

Characteristics	Low-risk group n = 75	High-risk group n = 123
Coagulation	40.0% (30)	32.5% (40)
Kidneys	6.7% (5)	1.6% (2)
Brain	9.3% (7)	8.9% (11)
Lungs	1.3% (1)	0.8% (1)
Circulation	5.3% (4)	1.6% (2)
ACLF grade		
ACLF-1	60.0% (45)	61.0% (75)
ACLF-2	32.0% (24)	30.1% (37)
ACLF-3	8.0% (6)	8.9% (11)
Severity score		
COSSH-ACLF IIs	7.3 [6.8, 8.1]	7.1 [6.5, 8.0]*
COSSH-ACLFs	6.4 [5.7, 6.9]	6.2 [5.7, 6.8]
CLIF-C ACLFs	43.3 [38.0, 49.1]	42.5 [36.6, 48.2]
MELD-Na	28.5 [25.2, 30.8]	27.0 [25.4, 29.9]
MELD	27.0 [24.1, 30.0]	26.1 [24.3, 28.9]
CTP	11.0 [10.0, 12.0]	11.0 [10.0, 12.0]
Transplant-free mortality (No. deceased patients)		
28-day	10.7% (8)	46.3% (57)**
90-day	18.7% (14)	58.5% (72)**

NOTE. Data are expressed as the mean \pm standard deviation (SD), median (p25, p75) or percentage (number of patients).

COSSH-ACLF IIs, COSSH-ACLF II score; COSSH-ACLFs, COSSH-ACLF score; CLIF-C ACLFs, CLIF Consortium ACLF score; MELDs, Model for End-stage Liver Disease score; MELD-Na, MELD-sodium score; CTP, Child-Turcotte-Pugh score; THBS1, thrombospondin 1. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ for comparisons between the groups.

GO:0071675	regulation of mononuclear cell migration	0.02	[SERPINE1, THBS1, TNF]
GO:0072577	endothelial cell apoptotic process	0.00	[CC12, IL10, SERPINE1, THBS1, TNF]
GO:1504707	positive regulation of vascular smooth muscle cell proliferation	0.01	[IL10, MMP9, TNF]
GO:1504894	positive regulation of receptor signaling pathway via STAT	0.00	[IL10, IL6, LIF, TNF, TNFSF18]
GO:1590889	cellular response to chemokine	0.00	[CC12, CCL22, CCL7, CCL8, CCR7]
GO:2000401	regulation of lymphocyte migration	0.03	[CXCL12, CCL12]
GO:0002690	positive regulation of leukocyte chemotaxis	0.00	[CCL7, CCR7, IL6, SERPINE1, THBS1]
GO:0022712	regulation of B cell mediated immunity	0.00	[CXCL12, CXCL13, IL10, TNF]
GO:0022792	negative regulation of peptide secretion	0.00	[ADRA2A, CACPP1, CCR7, FCGR2B, IL10, INHBB, PTGER4, TNF]
GO:0005509	membrane protein ectodomain proteolysis	0.01	[ADRA2A, IL10, TNF]
GO:0008059	chemokine activity	0.00	[CXCL12, CCL2, CCL22, CCL7, CCL8]
GO:0011638	zymogen activation	0.02	[CXCL12, SERPINE1, THBS1]
GO:0046425	regulation of receptor signaling pathway via JAK-STAT	0.00	[CDK5R1, IL10, IL6, LIF, TNF, TNFSF18]
GO:0051043	regulation of membrane protein ectodomain proteolysis	0.00	[ADRA2A, IL10, TNF]
GO:1504994	regulation of epithelial cell apoptotic process	0.00	[IL6, SERPINE1, THBS1, TNF]
GO:1504994	regulation of leukocyte adhesion to vascular endothelial cell	0.00	[IL6, SELL, TNF]
GO:0072677	eosinophil migration	0.00	[CCL2, CCL7, CCL8, PTGER4]
GO:0030593	neutrophil chemotaxis	0.01	[CCL2, CCL22, CCL7, CCL8, CCR7]
GO:0046427	positive regulation of receptor signaling pathway via JAK-STAT	0.00	[IL10, IL6, LIF, TNF, TNFSF18]
GO:0046245	eosinophil chemotaxis	0.00	[CCL2, CCL7, CCL8]
GO:0050709	negative regulation of protein secretion	0.00	[ADRA2A, CCR7, FCGR2B, IL10, INHBB, PTGER4, TNF]
GO:0050710	negative regulation of cytokine secretion	0.00	[CCR7, FCGR2B, IL10, PTGER4, TNF]
GO:1504037	positive regulation of epithelial cell apoptotic process	0.00	[CCL2, IL6, THBS1]
GO:1504996	positive regulation of leukocyte adhesion to vascular endothelial cell	0.00	[IL6, SELL, TNF]
GO:0002889	regulation of immunoglobulin mediated immune response	0.00	[CXCL12, CXCL13, IL10, TNF]
GO:1502041	regulation of extrinsic apoptotic signaling pathway via death domain receptors	0.03	[IL6, SERPINE1, THBS1]
GO:2000311	regulation of endothelial cell apoptotic process	0.00	[CCL2, SERPINE1, THBS1, TNF]
GO:0043114	negative regulation of cysteine-type endopeptidase activity involved in apoptotic process	0.00	[IL6, PTGS2, TNF, THBS1, TNF]
GO:0070314	positive regulation of ERK1 and ERK2 cascade	0.00	[BMPK1, CCL2, CCL22, CCL7, CCL8, CCR7, FGF3, GPCR2, GPCR4, GPCR5, TNF]
GO:1503672	positive regulation of sprouting angiogenesis	0.01	[BMPK1, IL10, PTGS2]
GO:2000117	negative regulation of cysteine-type endopeptidase activity	0.00	[IL6, PTGS2, TNF, THBS1, TNF]

Table S8. The immune-related biological processes for top 200 DEGs which were both differentially expressed in the comparisons of ACLF vs. LC and ACLF vs. NC

GO ID	GO Term	Term	PValue	Associated Genes Found
GO:0070664	negative regulation of leukocyte proliferation		0.0142	[CCL8, FCGR2B, GPNMB, IL10]
GO:0002251	organ or tissue specific immune response		0.0091	[C17orf99, GCNT3, IL6]
GO:0002381	immunoglobulin production involved in immunoglobulin mediated immune response		0.0308	[C17orf99, GCNT3, IL10]
GO:0070741	response to interleukin-6		0.0097	[C1QTNF4, FGF23, IL6]
GO:0071354	cellular response to interleukin-6		0.0074	[C1QTNF4, FGF23, IL6]
GO:0002286	T cell activation involved in immune response		0.0048	[CLEC4D, FCGR2B, IL6, PTGER4, TNFSF18]
GO:0002292	T cell differentiation involved in immune response		0.0332	[CLEC4D, IL6, PTGER4]
GO:0002701	negative regulation of production of molecular mediator of immune response		0.0069	[FCGR2B, IL10, TNF]
GO:0002761	regulation of myeloid leukocyte differentiation		0.0085	[CARTPT, FSTL3, LIF, SLC9B2, TNF]
GO:0002763	positive regulation of myeloid leukocyte differentiation		0.0213	[LIF, SLC9B2, TNF]
GO:0061756	leukocyte adhesion to vascular endothelial cell		0.0011	[IL6, SELE, SELL, TNF]
GO:0050901	leukocyte tethering or rolling		0.004	[SELE, SELL, TNF]
GO:1904994	regulation of leukocyte adhesion to vascular endothelial cell		0.003	[IL6, SELE, TNF]
GO:1904996	positive regulation of leukocyte adhesion to vascular endothelial cell		0.0013	[IL6, SELE, TNF]
GO:1905517	macrophage migration		0.0274	[CCL2, EDNRB, THBS1]
GO:0048246	macrophage chemotaxis		0.0145	[CCL2, EDNRB, THBS1]
GO:0002468	dendritic cell antigen processing and presentation		0.0002	[CCR7, FCGR2B, THBS1]
GO:0002577	regulation of antigen processing and presentation		0.0015	[CCR7, FCGR2B, THBS1]
GO:0002604	regulation of dendritic cell antigen processing and presentation		0.0002	[CCR7, FCGR2B, THBS1]
GO:0032615	interleukin-12 production		0.0264	[CCR7, IL10, THBS1]
GO:0002544	chronic inflammatory response		0.0009	[IL10, THBS1, TNF]
GO:0032655	regulation of interleukin-12 production		0.0243	[CCR7, IL10, THBS1]
GO:0032695	negative regulation of interleukin-12 production		0.0009	[CCR7, IL10, THBS1]
GO:0002688	regulation of leukocyte chemotaxis		0.0025	[CCL2, CCL7, CCR7, IL6, SERPINE1, THBS1]
GO:0150076	neuroinflammatory response		0.0062	[IL6, MMP3, PTGS2, TNF]
GO:0150077	regulation of neuroinflammatory response		0.0004	[IL6, MMP3, PTGS2, TNF]
GO:0002675	positive regulation of acute inflammatory response		0.0003	[CCR7, IL6, PTGS2, TNF]
GO:0031649	heat generation		0.0009	[EDNRB, PTGS2, TNF]
GO:0001660	fever generation		0.0002	[EDNRB, PTGS2, TNF]
GO:0002673	regulation of acute inflammatory response		1E-05	[CCR7, EDNRB, FCGR2B, IL6, PTGS2, TNF]
GO:0006953	acute-phase response		0.0035	[EDNRB, IL6, PTGS2, TNF]
GO:0031620	regulation of fever generation		0.0001	[EDNRB, PTGS2, TNF]
GO:0032715	negative regulation of interleukin-6 production		0.0116	[IL10, IL6, TNF]
GO:0002526	acute inflammatory response		0.0003	[CCR7, EDNRB, FCGR2B, IL6, PTGS2, TNF, TREM1]
GO:0002718	regulation of cytokine production involved in immune response		0.0181	[IL10, IL6, NR4A3, TNF]
GO:0002700	regulation of production of molecular mediator of immune response		0.0015	[C17orf99, CGAS, FCGR2B, IL10, IL6, NR4A3, TNF]
GO:0042116	macrophage activation		0.0038	[FCGR2B, IL10, IL6, THBS1, TNF]
GO:0002437	inflammatory response to antigenic stimulus		0.0039	[CCR7, FCGR2B, IL10, TNF]
GO:0032637	interleukin-8 production		0.0123	[FCN1, IL10, SERPINE1, TNF]
GO:0030595	leukocyte chemotaxis		9E-06	[C10orf99, CCL2, CCL22, CCL7, CCL8, CCR7, CH25H, EDNRB, IL10, IL6, SERPINE1, THBS1]
GO:0071674	mononuclear cell migration		9E-06	[CCL2, CCL22, CCL7, CCL8, IL6, SERPINE1, THBS1, TNF]
GO:0072676	lymphocyte migration		0.0002	[C10orf99, CCL2, CCL22, CCL7, CCL8, CCR7, CH25H]
GO:0097529	myeloid leukocyte migration		0.0002	[CCL2, CCL22, CCL7, CCL8, CCR7, EDNRB, IL6, PTGER4, SERPINE1, THBS1]
GO:0002637	regulation of immunoglobulin production		0.0001	[C17orf99, CGAS, FCGR2B, IL10, IL6, TNF]
GO:0002685	regulation of leukocyte migration		3E-06	[C10orf99, CCL2, CCL7, CCL8, CCR7, IL6, PTGER4, SELE, SERPINE1, THBS1, TNF, TNFSF18]
GO:0002922	positive regulation of humoral immune response		3E-05	[ACOD1, CCR7, FCGR2B, TNF]
GO:0043030	regulation of macrophage activation		0.0035	[FCGR2B, IL10, IL6, THBS1]
GO:0002687	positive regulation of leukocyte migration		2E-05	[CCL7, CCL8, CCR7, IL6, SELE, SERPINE1, THBS1, TNF, TNFSF18]
GO:0002861	regulation of inflammatory response to antigenic stimulus		0.0002	[CCR7, FCGR2B, IL10, TNF]
GO:0032677	regulation of interleukin-8 production		0.0085	[FCN1, IL10, SERPINE1, TNF]
GO:0002548	monocyte chemotaxis		8E-05	[CCL2, CCL22, CCL7, CCL8, IL6, SERPINE1]
GO:0048247	lymphocyte chemotaxis		6E-05	[C10orf99, CCL2, CCL22, CCL7, CCL8, CH25H]
GO:0097530	granulocyte migration		0.0012	[CCL2, CCL22, CCL7, CCL8, CCR7, PTGER4, THBS1]
GO:0032757	positive regulation of interleukin-8 production		0.0204	[FCN1, SERPINE1, TNF]
GO:0071621	granulocyte chemotaxis		0.0028	[CCL2, CCL22, CCL7, CCL8, CCR7, THBS1]
GO:0071675	regulation of mononuclear cell migration		0.0195	[SERPINE1, THBS1, TNF]
GO:2000401	regulation of lymphocyte migration		0.0264	[C10orf99, CCL2, CCL7]
GO:0002690	positive regulation of leukocyte chemotaxis		0.0035	[CCL7, CCR7, IL6, SERPINE1, THBS1]
GO:0002712	regulation of B cell mediated immunity		0.0031	[C17orf99, FCGR2B, IL10, TNF]
GO:0072677	eosinophil migration		0.0002	[CCL2, CCL7, CCL8, PTGER4]
GO:0030593	neutrophil chemotaxis		0.0052	[CCL2, CCL22, CCL7, CCL8, CCR7]
GO:0048245	eosinophil chemotaxis		0.0019	[CCL2, CCL7, CCL8]
GO:0002889	regulation of immunoglobulin mediated immune response		0.0031	[C17orf99, FCGR2B, IL10, TNF]

Table S9. The apoptosis-related biological processes for top 200 DEGs which were both differentially expressed in the comparisons of ACLF vs. LC and ACLF vs. NC

GO ID	GO Term	Term	PValue	Associated Genes Found
GO:0043277	apoptotic cell clearance		0.0137	[CCL2, FCN1, THBS1]
GO:1904037	positive regulation of epithelial cell apoptotic process		0.0047	[CCL2, IL6, THBS1]
GO:0072577	endothelial cell apoptotic process		0.0003	[CCL2, IL10, SERPINE1, THBS1, TNF]
GO:1904035	regulation of epithelial cell apoptotic process		0.0019	[CCL2, IL6, SERPINE1, THBS1, TNF]
GO:2000351	regulation of endothelial cell apoptotic process		0.0017	[CCL2, SERPINE1, THBS1, TNF]
GO:0043277	apoptotic cell clearance		0.0137	[CCL2, FCN1, THBS1]
GO:1904019	epithelial cell apoptotic process		0.0009	[CCL2, IL10, IL6, SERPINE1, THBS1, TNF]
GO:1904035	regulation of epithelial cell apoptotic process		0.0019	[CCL2, IL6, SERPINE1, THBS1, TNF]
GO:1904037	positive regulation of epithelial cell apoptotic process		0.0047	[CCL2, IL6, THBS1]
GO:1902041	regulation of extrinsic apoptotic signaling pathway via death domain receptors		0.0332	[IL6, SERPINE1, THBS1]
GO:0008625	extrinsic apoptotic signaling pathway via death domain receptors		0.017	[IL6, SERPINE1, THBS1, TNF]
GO:0043154	negative regulation of cysteine-type endopeptidase activity involved in apoptotic process		0.0029	[IL6, PTGS2, SFN, THBS1, TNF]
GO:0043154	negative regulation of cysteine-type endopeptidase activity involved in apoptotic process		0.0029	[IL6, PTGS2, SFN, THBS1, TNF]
GO:0071887	leukocyte apoptotic process		0.0065	[CCR7, IL10, IL6, NR4A3, PTCRA]
GO:1903202	negative regulation of oxidative stress-induced cell death		0.0027	[IL10, IL6, NR4A3, PYCR1]
GO:0036474	cell death in response to hydrogen peroxide		0.0004	[IL10, IL6, NR4A3, PYCR1]
GO:1903205	regulation of hydrogen peroxide-induced cell death		0.0002	[IL10, IL6, NR4A3, PYCR1]
GO:2000106	regulation of leukocyte apoptotic process		0.0137	[CCR7, IL10, NR4A3, PTCRA]
GO:1903206	negative regulation of hydrogen peroxide-induced cell death		0.0001	[IL10, IL6, NR4A3, PYCR1]
GO:0036473	cell death in response to oxidative stress		0.0033	[IL10, IL6, MMP3, NR4A3, PYCR1]
GO:0071887	leukocyte apoptotic process		0.0065	[CCR7, IL10, IL6, NR4A3, PTCRA]
GO:1903201	regulation of oxidative stress-induced cell death		0.0012	[IL10, IL6, MMP3, NR4A3, PYCR1]
GO:1903202	negative regulation of oxidative stress-induced cell death		0.0027	[IL10, IL6, NR4A3, PYCR1]
GO:0036474	cell death in response to hydrogen peroxide		0.0004	[IL10, IL6, NR4A3, PYCR1]
GO:1903205	regulation of hydrogen peroxide-induced cell death		0.0002	[IL10, IL6, NR4A3, PYCR1]
GO:1903206	negative regulation of hydrogen peroxide-induced cell death		0.0001	[IL10, IL6, NR4A3, PYCR1]
GO:1904019	epithelial cell apoptotic process		0.0009	[CCL2, IL10, IL6, SERPINE1, THBS1, TNF]
GO:0008625	extrinsic apoptotic signaling pathway via death domain receptors		0.017	[IL6, SERPINE1, THBS1, TNF]
GO:0072577	endothelial cell apoptotic process		0.0003	[CCL2, IL10, SERPINE1, THBS1, TNF]
GO:1904035	regulation of epithelial cell apoptotic process		0.0019	[CCL2, IL6, SERPINE1, THBS1, TNF]
GO:1904037	positive regulation of epithelial cell apoptotic process		0.0047	[CCL2, IL6, THBS1]
GO:1902041	regulation of extrinsic apoptotic signaling pathway via death domain receptors		0.0332	[IL6, SERPINE1, THBS1]
GO:2000351	regulation of endothelial cell apoptotic process		0.0017	[CCL2, SERPINE1, THBS1, TNF]
GO:0043154	negative regulation of cysteine-type endopeptidase activity involved in apoptotic process		0.0029	[IL6, PTGS2, SFN, THBS1, TNF]