**Supplementary Materials**

**Virological response to nucleos(t)ide analogues treatment in chronic hepatitis B patients is associated with *Bacteroides*-dominant gut microbiome**

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**Table S1 HBV Cohort Characteristics.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Overall  (n=110) | Treatment-Naïve  (n=26) | On-Treatment  (n=84) | p  value |
| Age, years | 61.2 (57.3-65.7) | 59.5 (55.3-64.0) | 62.3 (57.4-66.9) | 0.0257 |
| Male, n (%) | 80 (72.7%) | 16 (61.5%) | 64 (76.2%) | 0.2065 |
| BMI, kg/m2 | 25.3 (23.2-28.3) | 25.1 (23.5-29.5) | 25.6 (23.0-28.2) | 0.8304 |
| Fasting blood glucose, mmol/L | 6.2 (5.3-7.6) | 5.8 (5.1-7.0) | 6.5 (5.3-7.7) | 0.1077 |
| HbA1c, % | 6.0 (5.4-7.0) | 5.8 (5.4-6.4) | 6.1 (5.4-7.12) | 0.2700 |
| Triglyceride, mmol/L | 1.1 (0.8-1.5) | 1.1 (0.8-1.6) | 1.1 (0.9-1.5) | 0.9009 |
| Total cholesterol, mmol/L | 4.2 (3.7-5.1) | 4.50 (3.8-5.0) | 4.15 (3.6-5.1) | 0.6154 |
| HDL-cholesterol, mmol/L | 1.3 (1.1-1.6) | 1.40 (1.1-1.7) | 1.30 (1.0-1.5) | 0.0668 |
| LDL-cholesterol, mmol/L | 2.4 (1.8-3.0) | 2.4 (2.0-2.8) | 2.35 (1.7-3.1) | 0.9846 |
| ALT, U/L | 29.0 (22.2-46.8) | 26.0(20.8-34.0) | 30.5 (23.0-51.0) | 0.1415 |
| AST, U/L | 29.5 (23.0-38.0) | 24.5 (20.0-29.3) | 32.0 (24.0-39.0) | 0.0020 |
| GGT, U/L | 35.5 (22.2-58.0) | 27.0 (17.8-38.8) | 37.0 (23.0-71.0) | 0.0289 |
| ALP, U/L | 67.0 (57.0-77.0) | 65.4 (55.5-77.0) | 67.0 (58.3-78.0) | 0.2858 |
| Albumin, g/L | 46.0 (44.0-48.0) | 47.0 (44.8-48.3) | 46.0 (44.0-48.0) | 0.8618 |
| Total Bilirubin, umol/L | 12.0 (9.0-14.0) | 10.0 (7.0-12.3) | 12.0 (9.0-17.0) | 0.0092 |
| Platelet, 109/L | 198.5 (152.2-250.5) | 232 (208-273) | 183.5 (140.3-238.5) | 0.0002 |
| CAP, dB/m | 285.5 (227.8-319.0) | 264.0 (221.3-332.3) | 285.5 (228.5-315.0) | 0.9373 |
| LSM, kPa | 5.9 (5.0-11.9) | 5.0 (4.3-5.6) | 9.4 (5.4-13.6) | 0.0002 |
| Plasma HBV DNA |  |  |  | 0.0006 |
| Undetectable | 50 (45.5 %) | 4 (15.4%) | 46 (54.8%) |  |
| Detectable | 60 (54.5 %) | 22 (84.6%) | 38 (45.2%) |  |
| Antiviral treatment (months) | |  | 98.1 (48.3-130.0) |  |

Numerical data were presented as median (interquartile range), and categorical variables were presented in n (%). A significant difference was compared between treatment-naïve HBV patients and those who were on-treatment. Undetectable plasma HBV DNA was defined as <4.4 IU/mL. CAP, controlled attenuation parameter; LSM, liver stiffness measurement; BMI, body mass index; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase.

Table S2 Fasting plasma bile acid in HBV patients with different microbial clusters.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Bile acid (nM)** | | | **c-*Bacteroides* (n=49)** | **c-*Blautia* (n=44)** | **c-*Prevotella* (n=17)** | | | |
|  |  | C4 | 131.49 (102.21-178.26) | 110.45 (90.15-157.42) |  | 108.55 (89.31-142.84) |  |  |
| Primary bile acids | CA | CA | 87.48 (34.02-137.04) | 40.25 (25.27-104.85) |  | 163.15 (54.48-550.46) | ^ |  |
| GCA | 198.89 (95.57-415.57) | 107.68 (60.25-463.35) |  | 507.91 (178.53-993.92) | ^ |  |
| TCA | 25.40 (4.95-52.47) | 14.58 (4.75-94.68) |  | 81.23 (15.84-271.28) |  |  |
| CDCA | CDCA | 368.34 (159.23-756.55) | 198.79 (73.93-513.54) | \* | 582.32 (204.98-1104.77) | ^ |  |
| GCDCA | 1074.45 (644.98-1892.92) | 623.52 (319.49-1962.14) |  | 1513.71 (647.43-3496.26) |  |  |
| TCDCA | 101.42 (48.77-207.92) | 80.40 (34.68-215.27) |  | 155.11 (83.53-578.74) |  |  |
| Secondary bile acids | DCA | DCA | 244.21 (109.66-596.07) | 326.95 (169.97-507.17) |  | 543.34 (344.90-912.19) | ^^ | ## |
| GDCA | 186.18 (42.91-421.98) | 170.11 (84.65-401.19) |  | 551.27 (192.06-911.28) |  |  |
| TDCA | 21.58 (3.42-57.31) | 26.77 (10.42-66.16) |  | 55.97 (32.40-192.78) |  | # |
| LCA | GLCA | 0.21 (0.00-5.09) | 2.32 (0.00-9.61) |  | 6.91 (3.07-17.46) |  | ## |
| TLCA | 0 (0-0) | 0 (0-0) |  | 0.00 (0.00-2.45) |  |  |
| UDCA | UDCA | 173.91 (74.92 - 420.32) | 62.93 (38.67-188.84) | \*\* | 112.62 (46.64-235.71) |  |  |
| GUDCA | 139.49 (81.56 - 322.49) | 48.60 (24.67-124.43) | \*\* | 75.26 (41.68-205.99) |  |  |
| TUDCA | 6.03 (3.36 - 14.76) | 3.85 (0.67-6.99) | \* | 4.05 (0.29-14.52) |  |  |
| MCA | TaMCA | 1.72 (0.16-6.07) | 3.01 (1.42-6.81) |  | 7.20 (4.75-18.12) | ^ | ## |
| TbMCA | 0.00 (0.00-1.22) | 0.24 (0.00-0.76) |  | 1.30 (0.13-2.14) |  |  |
| HCA | GHCA | 6.55 (3.04-9.41) | 4.06 (2.80-7.64) |  | 9.12 (6.19-14.44) | ^ |  |
| THCA | 0.65 (0.14-2.28) | 0.44 (0.18-1.53) |  | 2.49 (0.57-3.73) | ^ |  |
|  | ACA | 1.63 (0.00-6.83) | 0.70 (0.00-5.58) |  | 3.58 (0.00-9.43) |  |  |
| Composite data | | Total BA | 3289.73 (2055.42-5716.26) | 2591.52 (1212.56-4782.87) |  | 5294.99 (3307.71-9972.43) | ^^ |  |
| Conjugated BA | 1966.16 (1144.19-3417.45) | 1389.32 (642.73-3679.69) |  | 3554.04 (1712.11-6197.66) |  |  |
| Unconjugated BA | 1044.52 (610.44-1806.27) | 813.46 (496.15-1341.47) |  | 1462.59 (758.56-3144.57) | ^ |  |
| Primary BA | 2265.48 (1217.26-3509.71) | 1455.39 (648.46-3179.84) |  | 3751.21 (1883.34-7601.49) | ^^ |  |
| Secondary BA | 964.75 (583.64-1598.65) | 775.08 (523.85-1477.92) |  | 1657.41 (1139.57-2505.87) | ^ |  |

All data were expressed in median (IQR) in nanomolar (nM). Post hoc pairwise comparison between multiple groups was assessed by the Dunn’s test with Benjamini-Hochberg false discovery rate (FDR) correction after the Kruskal-Wallis test. \* p\_{FDR} <0.05, \*\* p\_{FDR} <0.01 for c-*Bacteroides* vs c-*Blautia*; ^ p\_{FDR} <0.05, ^^ p\_{FDR} <0.01 for c-*Blautia* vs c-*Prevotella*; # p\_{FDR} <0.05, ## p\_{FDR} <0.01 for c-*Bacteroides* vs c-*Prevotella*.

Total BA was the sum of all individual bile acid listed in the table: BA, bile acid; CA, cholic acid; GCA, glycocholic acid; TCA, taurocholic acid; CDCA, chenodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; DCA, deoxycholic acid; GDCA, glycodeoxycholic acid; TDCA, taurodeoxycholic acid; GLCA, glycolithocholic acid; TLCA, taurolithocholic acid; UDCA, ursodeoxycholic acid; GUDCA, glycoursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; TaMCA, tauro-α-muricholic acid; TbMCA, tauro-β-muricholic acid; GHCA, glycohyocholic acid; THCA, taurohyocholic acid; ACA, allocholic acid.

**Table S3 Differential bacterial genera in clusters by LEfSe analysis.** c-*Bacteroides* bacterial communities associated positively with undetectable HBV DNA levels.

|  |  |  |  |
| --- | --- | --- | --- |
| Microbial genera | Clusters | LDA | FDR-p |
| *Bacteroides* | c-*Bacteroides* | 5.33 | 0.00 |
| *Flavonifractor* | c-*Bacteroides* | 3.21 | 0.00 |
| *Ruminococcus gnavus* | c-*Bacteroides* | 3.07 | 0.04 |
| *Lachnoclostridium* | c-*Bacteroides* | 3.62 | 0.00 |
| *Phascolarctobacterium* | c-*Bacteroides* | 3.58 | 0.01 |
| *Fusobacterium* | c-*Bacteroides* | 4.19 | 0.00 |
| *Parabacteroides* | c-*Bacteroides* | 4.08 | 0.01 |
| *Bilophila* | c-*Bacteroides* | 3.35 | 0.00 |
| *Oscillospiraceae UCG\_005* | c-*Blautia* | 3.32 | 0.00 |
| *Oscillospiraceae UCG\_002* | c-*Blautia* | 3.88 | 0.00 |
| *Roseburia* | c-*Blautia* | 3.54 | 0.01 |
| *Butyricicoccus* | c-*Blautia* | 3.16 | 0.00 |
| *Oscillospiraceae UCG\_003* | c-*Blautia* | 3.05 | 0.00 |
| *Odoribacter* | c-*Blautia* | 3.50 | 0.01 |
| *Anaerovoracaceae XIIIAD3011* | c-*Blautia* | 3.58 | 0.00 |
| *Lachnospiraceae ND3007* | c-*Blautia* | 3.23 | 0.00 |
| *Oscillibacter* | c-*Blautia* | 3.28 | 0.00 |
| *Ruminococcus torques* | c-*Blautia* | 3.61 | 0.02 |
| *Coprobacter* | c-*Blautia* | 3.37 | 0.00 |
| *Coprococcus* | c-*Blautia* | 3.42 | 0.01 |
| *Incertae Sedis* | c-*Blautia* | 3.03 | 0.05 |
| *Anaerostipes* | c-*Blautia* | 3.32 | 0.00 |
| *Alistipes* | c-*Blautia* | 4.21 | 0.01 |
| *Monoglobus* | c-*Blautia* | 3.07 | 0.00 |
| *Clostridia UCG\_014* | c-*Blautia* | 3.63 | 0.00 |
| *Negativibacillus* | c-*Blautia* | 3.00 | 0.02 |
| *Dorea* | c-*Blautia* | 3.21 | 0.00 |
| *Bifidobacterium* | c-*Blautia* | 3.93 | 0.00 |
| *Eubacterium hallii* | c-*Blautia* | 3.45 | 0.00 |
| *Romboutsia* | c-*Blautia* | 3.21 | 0.00 |
| *Eubacterium coprostanoligenes* | c-*Blautia* | 3.58 | 0.00 |
| *Lachnospiraceae FCS020* | c-*Blautia* | 3.71 | 0.00 |
| *Oscillospiraceae* unclassified | c-*Blautia* | 3.31 | 0.01 |
| *Hungatella* | c-*Blautia* | 3.62 | 0.03 |
| *Lachnospiraceae NK4A136* | c-*Blautia* | 3.42 | 0.00 |
| *Muribaculaceae* unclassified | c-*Blautia* | 3.41 | 0.00 |
| *Christensenellaceae R\_7* | c-*Blautia* | 3.37 | 0.00 |
| *Ruminococcaceae UBA1819* | c-*Blautia* | 3.13 | 0.01 |
| *Dialister* | c-*Blautia* | 3.61 | 0.00 |
| *Oscillospiraceae NK4A214* | c-*Blautia* | 3.26 | 0.00 |
| *Subdoligranulum* | c-*Blautia* | 3.68 | 0.00 |
| *Erysipelotrichaceae UCG\_003* | c-*Blautia* | 3.30 | 0.01 |
| *Collinsella* | c-*Blautia* | 2.99 | 0.02 |
| *Eubacterium eligens* | c-*Blautia* | 3.45 | 0.01 |
| *Blautia* | c-*Blautia* | 3.92 | 0.00 |
| *Prevotella\_9* | c-*Prevotella* | 5.29 | 0.00 |
| *Clostridium sensu stricto 1* | c-*Prevotella* | 3.63 | 0.00 |

Differential gut bacterial genera between three clusters determined by the LEfSe with a threshold of log LDA score greater than 3 and FDR smaller than 0.05. The strategy for multi-group comparison was set to be “One-against-all (less stringent)” indicating that the bacterial taxa in at least one group were significantly different from all others. Only bacterial taxa of genus level were shown in the table.

**Table S4 Clinical characteristics in treatment-naïve HBV patients with different gut microbial clusters.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | c-*Bacteroides* (n=11) | Non-c-*Bacteroides* (n=15)  *c-Blautia (n=13) and c-Prevotella (n=2)* | | p |
| Gender |  |  | | 1.000 |
| Male | 7 (63.6%) | 9 (60.0%) | |  |
| Female | 4 (36.4%) | 6 (40.0%) | |  |
| Age (years) | 59.2 (54.0-63.9) | 59.7 (55.5-64.2) | | 0.829 |
| Steatosis |  |  | | 1.000 |
| Non-Steatosis | 5 (45.5%) | 8 (53.3%) | |  |
| Steatosis | 6 (54.5%) | 7 (46.7%) | |  |
| Fibrosis |  |  | | 0.356 |
| No advanced liver fibrosis (<F3) | 10 (90.9%) | 11 (73.3%) | |  |
| Advanced liver fibrosis (F3/4) | 1 (9.1%) | 4 (26.7%) | |  |
| Plasma HBV DNA | | |  | 0.614 |
| Undetectable | 1 (9.1%) | 3 (20.0%) | |  |
| Detectable | 10 (90.9%) | 12 (80.0%) | |  |
| BMI (kg/m2) | 25.3 (23.5-28.3) | 25.1 (23.5-32.1) | | 0.821 |
| CAP (dB/m) | 287.0 (228.0-354.0) | 241.0 (216.0-325.0) | | 0.515 |
| Liver Stiffness (kPa) | 4.8 (3.9-15.0) | 5.5 (4.3-9.0) | | 0.123 |
| Fasting blood glucose (mmol/L) | 6.0 (5.5-7.3) | 5.5 (4.7-7.0) | | 0.231 |
| HbA1c (%) | 5.7 (5.4-7.2) | 5.8 (5.1-6.3) | | 0.404 |
| Triglyceride (mmol/L) | 1.1 (0.8-1.7) | 1.1 (0.8-1.5) | | >0.999 |
| Total cholesterol (mmol/L) | 4.2 (3.8-4.8) | 4.5 (3.7-5.2) | | 0.711 |
| HDL-cholesterol (mmol/L) | 1.3 (1.1-1.7) | 1.4 (1.2-1.8) | | 0.547 |
| LDL-cholesterol (mmol/L) | 2.3 (2.1-2.7) | 2.5 (1.5-3.0) | | 0.692 |
| ALT (U/L) | 25.0 (20.0-34.0) | 27.0 (22.0-42.0) | | 0.404 |
| AST (U/L) | 21.0 (18.0-29.0) | 25.0 (22.0-30.0) | | 0.274 |
| GGT(U/L) | 28.0 (17.0-38.0) | 25.0 (18.0-41.0) | | 0.750 |
| ALP (U/L) | 56.0 (47.0-85.0) | 65.0 (57.0-77.0) | | 0.450 |
| Albumin (g/L) | 46.0 (44.0-47.0) | 47.0 (45.0-49.0) | | 0.233 |
| Total Bilirubin (μmol/L) | 0.0 (8.0-12.0) | 10.0 (6.0-13.0) | | 0.691 |
| Platelet (x 109/L) | 252.0 (220.0-276.0) | 223.0 (185.0-272.0) | | 0.131 |

**Table S5 Association of clinical parameters with gut microbial clusters in HBV patients by a multinomial logistic regression analysis.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | c-*Bacteroides vs* c-*Blautia* | | | | c-*Bacteroides vs* c-*Prevotella* | | | | c-*Blautia* vs c-*Prevotella* | | |
| Factors | Odds ratio (95%CI) | | | p | | Odds ratio (95%CI) | | p | Odds ratio (95%CI) | | p |
| **On-Treatment** | | | | | | | | | | | |
| Albumin | 1.02 | | (0.88-1.18) | 0.80 | | 1.29 | (1.08-1.53) | 0.00\* | 1.26 | (1.06-1.51) | 0.01\* |
| ALT | 1.01 | | (0.99-1.03) | 0.34 | | 1.06 | (1.01-1.12) | 0.02\* | 1.05 | (1.00-1.11) | 0.04\* |
| Undetectable HBV-DNA | 3.40 | | (1.25-9.24) | 0.02\* | | 3.68 | (1.06-12.83) | 0.04\* | 1.08 | (0.31-3.80) | 0.90 |
| Advanced Fibrosis | 0.45 | | (0.17-1.24) | 0.12 | | 2.22 | (0.64-7.74) | 0.21 | 4.89 | (1.30-18.38) | 0.02\* |
| HemoglobinA1c | 1.53 | | (0.99-2.35) | 0.06 | | 1.05 | (0.65-1.69) | 0.84 | 0.69 | (0.40-1.17) | 0.17 |
| **Treatment-Naïve** | | | | | | | | | | | |
| Albumin | 0.88 | (0.61-1.26) | | 0.49 | | 0.53 | (0.19-1.53) | 0.24 | 0.61 | (0.21-1.72) | 0.35 |
| ALT | 0.98 | (0.92-1.05) | | 0.65 | | 0.97 | (0.87-1.08) | 0.63 | 0.99 | (0.89-1.10) | 0.83 |
| Undetectable HBV-DNA | 0.55 | (0.04-7.04) | | 0.65 | | 0.10 | (0-30.15.00) | 0.19 | 0.18 | (0.01-4.26) | 0.29 |
| Advanced Fibrosis | 0.33 | (0.03-3.78) | | 0.38 | | 0.10 | (0.00-3.15) | 0.19 | 0.30 | (0.01-6.37) | 0.44 |
| HemoglobinA1c | 2.09 | (0.77-5.70) | | 0.15 | | 1.54 | (0.29-8.13) | 0.61 | 0.73 | (0.13-4.25) | 0.73 |

Table S6 Multivariate logistic regression for the association with undetectable HBV DNA.

|  |  |  |  |
| --- | --- | --- | --- |
| Independent variables | Odds ratio | (95%CI) | p |
| Gut microbiota |  |  |  |
| c-*Bacteroides* vs non- c-*Bacteroides* | 5.66 | (2.06 - 17.28) | 0.001 |
| Fibrosis |  |  |  |
| Advanced vs No advanced liver fibrosis | 1.61 | (0.61 - 4.43) | 0.341 |
| HemoglobinA1C | 0.68 | (0.44 - 1.02) | 0.070 |
| ALT | 0.99 | (0.96 - 1.00) | 0.127 |
| Albumin | 0.99 | (0.88 - 1.11) | 0.802 |

Multivariate logistic regression was used to quantify the association between HBV-DNA outcome and gut microbiota cluster in on-treatment HBV patients, while adjusting for potential covariates.

**Table S7 Comparison of fasting plasma bile acid concentration between no advanced fibrosis vs advanced fibrosis under different HBV-DNA subgroups.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Undetectable HBV DNA** | | | | | **Detectable HBV DNA** | | | | |
|  |  | **Bile acid (nM)** | No advanced liver fibrosis  (n=20) | | Advanced liver fibrosis  (n=26) | | p | No advanced liver fibrosis  (n=17) | | Advanced liver fibrosis  (n=21) | | p |
|  |  | C4 | 128.69 | (99.05-154.24) | 148.22 | (94.69-236.10) | 0.26 | 108.55 | (84.86-137.80) | 102.13 | (91.68-123.30) | 0.74 |
| Primary bile acids | CA | CA | 46.55 | (29.03-162.24) | 104.49 | (63.14-196.02) | 0.046 | 54.48 | (29.54-144.99) | 73.28 | (17.37-205.97) | 1.00 |
| GCA | 71.46 | (37.92-255.64) | 393.77 | (140.21-1380.12) | <0.01 | 136.22 | (101.51-335.03) | 349.63 | (166.70-661.04) | 0.16 |
| TCA | 10.76 | (1.57-33.36) | 37.99 | (14.17-188.34) | 0.06 | 20.01 | (7.95-55.90) | 78.90 | (23.74-186.29) | 0.07 |
| CDCA | CDCA | 218.09 | (64.80-501.63) | 636.19 | (318.86-955.56) | 0.01 | 317.91 | (89.31-693.76) | 500.55 | (94.44-627.79) | 0.95 |
| GCDCA | 572.15 | (280.74-919.60) | 1828.98 | (923.50-4355.31) | <0.01 | 883.63 | (586.94-1037.98) | 1320.66 | (785.10-2914.66) | 0.20 |
| TCDCA | 57.52 | (24.38-126.57) | 152.44 | (69.20-387.82) | <0.01 | 82.17 | (51.13-143.67) | 255.15 | (86.67-530.21) | 0.09 |
| Secondary bile acids | DCA | DCA | 262.12 | (153.10-684.72) | 304.78 | (71.42-684.27) | 0.72 | 647.78 | (324.02-752.35) | 298.29 | (63.73-459.02) | 0.01 |
| GDCA | 140.56 | (67.54-315.20) | 264.49 | (9.29-934.02) | 0.33 | 259.92 | (145.64-381.52) | 327.02 | (121.82-912.84) | 0.89 |
| TDCA | 23.63 | (4.86-33.74) | 29.55 | (2.57-91.47) | 0.53 | 32.40 | (18.60-79.48) | 76.00 | (10.19-179.97) | 0.92 |
| LCA | GLCA | 0.28 | (0.00-5.37) | 2.53 | (0.00-12.88) | 0.28 | 3.07 | (0.81-6.60) | 5.87 | (0.33-10.06) | 0.83 |
| TLCA | 0.00 | (0.00-0.00) | 0.00 | (0.00-1.09) | 0.15 | 0.00 | (0.00-0.00) | 0.00 | (0.00-1.81) | 0.26 |
| UDCA | UDCA | 72.17 | (48.13-141.09) | 200.29 | (72.93-412.10) | 0.054 | 115.83 | (68.69-253.25) | 89.64 | (61.07-212.12) | 0.52 |
| GUDCA | 74.06 | (22.31-146.28) | 168.05 | (75.05-406.79) | <0.01 | 86.58 | (43.53-231.22) | 104.58 | (43.06-304.53) | 0.98 |
| TUDCA | 3.42 | (0.00-9.41) | 6.84 | (3.19-18.98) | 0.04 | 4.54 | (2.11-12.65) | 6.16 | (2.33-24.90) | 0.68 |
| MCA | TaMCA | 4.31 | (0.83-12.74) | 2.72 | (0.00-6.31) | 0.26 | 4.52 | (1.64-10.68) | 4.23 | (1.61-24.05) | 0.95 |
| TbMCA | 0.37 | (0.02-1.35) | 0.00 | (0.00-1.06) | 0.15 | 0.31 | (0.00-1.59) | 0.80 | (0.05-3.74) | 0.28 |
| HCA | GHCA | 6.18 | (2.02-11.04) | 5.95 | (3.39-11.75) | 0.40 | 7.00 | (3.28-12.56) | 7.47 | (2.64-10.60) | 0.86 |
| THCA | 0.42 | (0.08-2.56) | 0.53 | (0.00-2.55) | 0.93 | 0.66 | (0.39-3.28) | 1.60 | (0.47-3.73) | 0.47 |
|  | ACA | 1.80 | (0.12-7.47) | 1.88 | (0.00-6.60) | 0.95 | 0.51 | (0.00-4.27) | 3.58 | (0.00-8.47) | 0.20 |
| Composite data | Total BA | | 2441.77 | (1408.52-4218.52) | 4533.36 | (2853.97-10156.50) | <0.01 | 2693.82 | (1829.10-4455.22) | 3384.59 | (2631.89-5998.13) | 0.47 |
| Conjugated BA | | 1136.64 | (485.31-2407.99) | 3394.39 | (1681.34-7313.61) | <0.01 | 1712.11 | (1060.74-2124.04) | 2757.11 | (1490.32-5044.32) | 0.26 |
| Unconjugated BA | | 781.97 | (519.89-1725.06) | 1315.44 | (743.66-2132.66) | 0.07 | 1100.81 | (728.63-2000.05) | 1100.28 | (408.58-1566.72) | 0.57 |
| Conjugated BA ratio | | 0.63 | (0.40-0.75) | 0.65 | (0.56-0.80) | 0.16 | 0.57 | (0.45-0.73) | 0.79 | (0.55-0.89) | 0.16 |
| Primary BA | | 1344.52 | (833.50-2735.24) | 3492.16 | (2056.15-8341.53) | <0.01 | 1554.35 | (1088.43-2998.27) | 2467.21 | (1639.04-4726.23) | 0.12 |
| Secondary BA | | 760.96 | (459.79-1479.68) | 1267.87 | (609.40-2108.37) | 0.30 | 1139.57 | (758.27-1936.58) | 964.75 | (766.47-1869.59) | 0.63 |
| Primary BA ratio | | 0.63 | (0.44-0.71) | 0.73 | (0.66-0.86) | <0.01 | 0.63 | (0.51-0.67) | 0.70 | (0.64-0.82) | 0.04 |
| Primary  Conjugated BA | | 813.42 | (380.43-41498.87) | 2654.80 | (1348.29-6553.60) | <0.01 | 1170.33 | (832.23-1431.64) | 1948.69 | (1132.90-4027.35) | 0.15 |
| Primary  Unconjugated BA | | 248.78 | (93.83-939.29) | 727.53 | (427.26-1150.52) | 0.15 | 368.14 | (1138.33-851.04) | 639.55 | (140.22-848.14) | 0.92 |
| Secondary  Conjugated BA | | 286.99 | (139.22-732.42) | 441.44 | (228.14-1300.27) | 0.09 | 457.95 | (328.75-636.25) | 663.80 | (214.68-1437.91) | 0.77 |
| Secondary  Unconjugated BA | | 406.42 | (286.26-839.81) | 508.40 | (284.54-995.77) | 0.72 | 708.37 | (384.24-1105.28) | 469.95 | (237.48-615.58) | 0.03 |

Concentration of bile acids was expressed in median (IQR) in nanomolar (nM). Ratio was expressed in the proportion (IQR) of the bile acids with respect to total BA. Comparison was made between MAFLD and non-MAFLD within the same HBV-DNA category by Mann-Whitney U test. Conjugated BA ratio was obtained by dividing conjugated BA by total BA; primary BA ratio was obtained by dividing primary BA by total BA; primary conjugated BA was the sum of GCA, TCA, GCDCA and TCDCA; primary unconjugated BA was the sum of CA and CDCA; secondary conjugated BA was the sum of GDCA, TDCA, GLCA, TLCA, GUDCA, TUDCA, GHCA, THCA, TaMCA and TbMCA; secondary unconjugated BA was the sum of DCA, UDCA and ACA

**Table S8 The receiver operating characteristic (ROC) analysis to evaluate the predictive efficacy of three distinct microbial clusters (C1, C2, and C3) and bacterial signatures for advanced fibrosis among NAs-treated CHB patients with undetectable HBV-DNA.**

|  |  |  |
| --- | --- | --- |
| Signatures | Area under the ROC curve (AUROC) | p-value |
| Bacteroides | 0.5423 | 0.6259 |
| Blautia | 0.5712 | 0.4123 |
| Prevotella\_9 | 0.5231 | 0.7903 |
| Escherichia Coli | 0.6404 | 0.1058 |
| Alistipes shahii | 0.7087 | 0.0162 |

**A close-up of a graph

Description automatically generated**

**Figure S1 The gut microbial signature regarding antiviral treatment. (A)** Shannon’s diversity index; **(B)** Chao 1 richness of gut microbiota; (**C**) Differentially abundant bacterial taxa between treatment-naïve and on-treatment HBV patients by Linear discriminant analysis (LDA) Effect Size analysis. **(D)** Proportion of *E. hallii* group; **(E)** Proportion *Ruminococcus*.

A diagram of a pathway

Description automatically generated with medium confidence

**Figure S2 L-tryptophan biosynthesis Pathway was enriched in on-treatment HBV patients with advanced fibrosis. (A)** L-tryptophan biosynthesis Pathway based on MetaCyc database; The relative abundance of gene **(B)** TrpA and **(C)** TrpB in feces of on-treatment HBV patients with or without advanced fibrosis by qPCR.

A close-up of a test results

Description automatically generated

**Figure S3 The effects of supernatants from *Escherichia coli* bacteria species and L-tryptophan on hepatic stellate cells (HSCs) activation.** Bacterial strains growing overnight in BHI broth were sub-cultured 1:50 in fresh BHI broth and grown for 24 h. Bacterial cultures were spun down at 11,000g for 2 min and the supernatant was carefully removed without disturbing the pellet. The supernatants were filtered through a 0.22-μM syringe filter to remove any remaining bacteria in the suspension. **(A)** human hepatic stellate cell (HSC)-LX-2 cell line was cultured with 1:3 (25%) or 1:1 dilution (50%) supernatant of spent media from *Escherichia coli* bacterial species **(B)** or with L-tryptophan (0, 10, 50, 100, 200, 400µM) for 24 hours. Western blot was used to evaluate the expression of α-SMA and COL1A1 after treatment.