**Supplemental Digital Content 5: Sensitivity analysis of serum bile acid profile of the HC and NAFLD groups using the analysis of covariance model with BMI and HOMA-IR as covariates**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | P-value |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | HC | HC | MF |
| Serum bile Acid(μM) | HC |  | NAFLD (MF) |  |  | NAFLD (AF) |  | vs | vs | vs |
| (n = 55) | 　 | 　 | (n = 52) | 　 | 　 | (n = 34) | 　 | AF | MF | AF |
| *Total BA* | 7.1  | ( | 5.4  | - | 8.7  | ) |  | 8.4  | ( | 7.0  | - | 9.9  | ) |  | 10.9  | ( | 9.1  | - | 12.7  | ) | 0.02  | 0.5  | 0.06  |
|  | Conj BA | 3.3  | ( | 2.1  | - | 4.4  | ) |  | 3.2  | ( | 2.3  | - | 4.2  | ) |  | 3.8  | ( | 2.6  | - | 5.0  | ) | 0.8  | 1  | 0.7  |
|  | Unconj BA | 3.8  | ( | 2.5  | - | 5.1  | ) |  | 5.2  | ( | 4.1  | - | 6.3  | ) |  | 7.1  | ( | 5.7  | - | 8.5  | ) | 0.01  | 0.3  | 0.07  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Primary BA | 3.0  | ( | 2.2  | - | 3.8  | ) |  | 3.9  | ( | 3.2  | - | 4.5  | ) |  | 5.2  | ( | 4.4  | - | 6.1  | ) | 0.003  | 0.3  | 0.02  |
|  | Secondary BA | 4.1  | ( | 2.9  | - | 5.3  | ) |  | 4.6  | ( | 3.6  | - | 5.6  | ) |  | 5.6  | ( | 4.4  | - | 6.9  | ) | 0.3  | 0.8  | 0.3  |
|  | S/P ratio | 1.6  | ( | 1.3  | - | 1.8  | ) |  | 1.4  | ( | 1.2  | - | 1.6  | ) |  | 1.2  | ( | 1.0  | - | 1.5  | ) | 0.4  | 0.6  | 0.7  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Total CA* | 0.6  | ( | 0.2  | - | 0.9  | ) |  | 1.1  | ( | 0.8  | - | 1.4  | ) |  | 1.9  | ( | 1.5  | - | 2.2  | ) | <.0001 | 0.06  | 0.002  |
|  | Unconj CA | 0.4  | ( | 0.1  | - | 0.7  | ) |  | 0.7  | ( | 0.5  | - | 1.0  | ) |  | 1.3  | ( | 1.0  | - | 1.6  | ) | 0.002  | 0.4  | 0.01  |
|  | Conj CA | 0.1  | ( | 0.0  | - | 0.2  | ) |  | 0.4  | ( | 0.3  | - | 0.5  | ) |  | 0.6  | ( | 0.5  | - | 0.7  | ) | <.0001 | 0.01  | 0.04  |
| *Total CDCA* | 2.4  | ( | 1.7  | - | 3.1  | ) |  | 2.7  | ( | 2.1  | - | 3.3  | ) |  | 3.4  | ( | 2.6  | - | 4.1  | ) | 0.2  | 0.8  | 0.3  |
|  | Unconj CDCA | 1.2  | ( | 0.7  | - | 1.7  | ) |  | 2.1  | ( | 1.7  | - | 2.6  | ) |  | 2.5  | ( | 1.9  | - | 3.1  | ) | 0.01  | 0.05  | 0.5  |
|  | Conj CDCA | 1.2  | ( | 0.7  | - | 1.8  | ) |  | 0.6  | ( | 0.2  | - | 1.1  | ) |  | 0.9  | ( | 0.3  | - | 1.5  | ) | 0.7  | 0.3  | 0.7  |
| *Total DCA* | 1.6  | ( | 0.7  | - | 2.5  | ) |  | 0.9  | ( | 0.1  | - | 1.6  | ) |  | 1.8  | ( | 0.8  | - | 2.8  | ) | 1.0  | 0.5  | 0.2  |
|  | Unconj DCA | 0.7  | ( | -0.1  | - | 1.6  | ) |  | 0.2  | ( | -0.5  | - | 1.0  | ) |  | 1.1  | ( | 0.2  | - | 2.0  | ) | 0.8  | 0.7  | 0.2  |
|  | Conj DCA | 0.9  | ( | 0.5  | - | 1.3  | ) |  | 0.6  | ( | 0.3  | - | 0.9  | ) |  | 0.7  | ( | 0.2  | - | 1.1  | ) | 0.8  | 0.6  | 1.0  |
| *Total LCA* | 0.8  | ( | 0.6  | - | 0.9  | ) |  | 1.0  | ( | 0.8  | - | 1.1  | ) |  | 1.4  | ( | 1.2  | - | 1.5  | ) | 0.0003  | 0.3  | 0.002  |
|  | Unconj LCA | 0.6  | ( | 0.4  | - | 0.7  | ) |  | 0.7  | ( | 0.5  | - | 0.8  | ) |  | 1.0  | ( | 0.9  | - | 1.2  | ) | 0.0005  | 0.6  | 0.0004  |
|  | Conj LCA | 0.2  | ( | 0.1  | - | 0.3  | ) |  | 0.3  | ( | 0.2  | - | 0.4  | ) |  | 0.3  | ( | 0.2  | - | 0.4  | ) | 0.1  | 0.1  | 0.9  |
| *Total UDCA* | 1.2  | ( | 0.8  | - | 1.7  | ) |  | 1.9  | ( | 1.5  | - | 2.2  | ) |  | 1.6  | ( | 1.2  | - | 2.1  | ) | 0.5  | 0.1  | 0.7  |
|  | Unconj UDCA | 0.7  | ( | 0.4  | - | 1.0  | ) |  | 1.1  | ( | 0.8  | - | 1.4  | ) |  | 0.9  | ( | 0.5  | - | 1.2  | ) | 0.9  | 0.2  | 0.4  |
|  | Conj UDCA | 0.5  | ( | 0.3  | - | 0.8  | ) |  | 0.7  | ( | 0.5  | - | 0.9  | ) |  | 0.8  | ( | 0.5  | - | 1.0  | ) | 0.5  | 0.5  | 1.0  |
| *Total HDCA* | 0.5  | ( | 0.2  | - | 0.7  | ) |  | 0.9  | ( | 0.7  | - | 1.1  | ) |  | 0.9  | ( | 0.6  | - | 1.2  | ) | 0.2  | 0.09  | 1.0  |
|  | Unconj HDCA | 0.2  | ( | 0.1  | - | 0.3  | ) |  | 0.3  | ( | 0.3  | - | 0.4  | ) |  | 0.3  | ( | 0.2  | - | 0.4  | ) | 0.8  | 0.1  | 0.4  |
|  | Conj HDCA | 0.3  | ( | 0.0  | - | 0.5  | ) | 　 | 0.6  | ( | 0.3  | - | 0.8  | ) | 　 | 0.6  | ( | 0.3  | - | 0.9  | ) | 0.3  | 0.3  | 1.0  |

Data are presented as mean ± 95% Confidence Interval.

AF, advanced fibrosis; BA, bile acid; BMI, body mass index; C4, 7α-hydroxy-4-cholesten-3-one; CA, cholic acid; CDCA, chenodeoxycholic acid; Conj, conjugated; DCA, deoxycholic acid; GCA, glycocholic acid; HC, healthy control; HDCA, hyodeoxycholic acid; HOMA-IR, homeostasis model assessment of insulin resistance; LCA, lithocholic acid; MF, mild fibrosis; NAFLD, nonalcoholic fatty liver disease; S/P, Secondary/primary; UDCA, ursodeoxycholic acid; Unconj, unconjugated