**Reduced hepatic steatosis is associated with higher risk of hepatocellular carcinoma in chronic hepatitis B infection**

Lung-Yi Mak, Rex Wan-Hin Hui, James Fung, Fen Liu, Danny Ka-Ho Wong, Ka-Shing Cheung, Man-Fung Yuen, Wai-Kay Seto

**Supplementary Appendix**

Supplementary table 1. Histological evaluation at the time of HCC diagnosis in 22 patients

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patient number | Time of TE before HCC diagnosis (months) | LS (kPa) | CAP (dB/m) | Type of imaging | Steatosis by imaging | Advanced fibrosis/cirrhosis by imaging | Steatosis by histology | Advanced fibrosis/ cirrhosis by histology |
| 802 | 27.4 | 7.4 | 127 | MRI | 0 | 0 | 0 | 0 |
| 1085 | 41.3 | 8.2 | 158 | CT | 0 | 1 | 0 | 1 |
| 1147 | 12.8 | 6.6 | 161 | CT | 0 | 0 | 0 | 0 |
| 61 | 15.2 | 12.2 | 165 | CT | 0 | 0 | 0 | 1 |
| 364 | 15.0 | 6.8 | 192 | CT | 0 | 0 | 0 | 0 |
| 593 | 7.9 | 8.2 | 199 | CT | 0 | 1 | 0 | 1 |
| 388 | 37.6 | 5.3 | 200 | CT | 0 | 0 | 0 | 0 |
| 697 | 22.2 | 5.9 | 206 | CT | 0 | 1 | 0 | 1 |
| 1656 | 13.6 | 9.5 | 209 | CT | 0 | 0 | 0 | 0 |
| 318 | 23.2 | 36.3 | 212 | CT | 0 | 1 | 1 (mild) | 1 |
| 83 | 23.1 | 22.8 | 227 | CT | 0 | 1 | 0 | 1 |
| 641 | 19.9 | 10.1 | 233 | CT | 0 | 1 | 0 | 1 |
| 2286 | 17.3 | 7.7 | 238 | USG | 1 | 0 | 0 | 0 |
| 1748 | 26.4 | 6.3 | 249 | CT | 0 | 0 | 0 | 0 |
| 554 | 13.6 | 4.3 | 254 | CT | 0 | 0 | 0 | 0 |
| 786 | 41.8 | 8 | 255 | CT | 0 | 1 | 1 (mild) | 0 |
| 49 | 37.7 | 9.1 | 276 | CT | 0 | 0 | 0 | 0 |
| 1543 | 26.6 | 16.2 | 277 | CT | 0 | 1 | 1 (mild) | 1 |
| 121 | 9.4 | 14.4 | 285 | CT | 0 | 1 | 0 | 1 |
| 96 | 25.7 | 17.3 | 343 | CT | 0 | 1 | 0 | 1 |
| 991 | 16.3 | 8.8 | 359 | MRI | 0 | 0 | 1 (severe) | 0 |
| 362 | 13.3 | 8.2 | 375 | CT | 0 | 0 | 1 (moderate) | 0 |

1=yes, 0=no, CAP: controlled attenuation parameter, HCC: hepatocellular carcinoma, LS: liver stiffness, TE: transient elastography

Text highlighted in red denotes presence of advanced liver fibrosis/ cirrhosis on either transient elastography or histology

Text highlighted in green denotes presence of hepatic steatosis on either transient elastography or histology

Supplementary table 2. Univariate analysis of risk factors for HCC development in all patients (N=2403)

|  |  |  |  |
| --- | --- | --- | --- |
|  | HCC (N=48) | No HCC (N=2355) | P value |
| Age | 62.9 (56.7 – 68.7) | 55.6 (46.5 – 62.8) | <0.001 |
| Gender (male) | 36 (75%) | 1300 (55.2%) | 0.004 |
| Body height (cm) | 163.5 (159.1 – 169) | 163.5 (157 – 169.5) | 0.775 |
| Body weight (kg) | 64.9 (57.3 – 70.6) | 64.7 (56.2 – 73) | 0.829 |
| Body mass index (kg/m2) | 24.0 (21.7 – 26.5) | 24.0 (21.7 – 26.9) | 0.828 |
| Waist circumference (cm) | 88 (84 – 93) | 86 (79 – 94) | 0.147 |
| Hip circumference (cm) | 96 (92 – 100) | 96 (92 – 101) | 0.940 |
| Systolic blood pressure (mmHg) | 139 (120 – 156) | 133 (121 – 147) | 0.303 |
| Diastolic blood pressure (mmHg) | 78 (72 – 88) | 79 (72 – 87) | 0.592 |
| Diabetes mellitus (yes) | 13/ 47 (27.7%) | 642/ 2228 (28.8%) | 0.505 |
| Glycated hemoglobin (%) | 5.7 (5.5 – 6.3) | 5.7 (5.3 – 6.4) | 0.435 |
| Dyslipidaemia (yes) | 21/ 48 (43.8%) | 1253/ 2343 (53.5%) | 0.191 |
| On lipid-lowering therapy (yes) | 10/48 (20.8%) | 520/2228 (23.3%) | 0.423 |
| On anti-diabetic therapy (yes) | 12/48 (25%) | 601/2228 (26.9%) | 0.832 |
| Platelet count (x100/L) | 127 (94 – 197) | 208 (167 – 249) | <0.001 |
| Albumin (gram/L) | 43 (39 – 46) | 45 (43 – 47) | <0.001 |
| Bilirubin (umol/L) | 11 (9 – 17) | 10 (7 – 13) | 0.004 |
| Alanine aminotransferase (U/L) | 28 (22 – 39) | 26 (19 – 36) | 0.149 |
| Aspartate aminotransferase (U/L) | 30 (24 – 40) | 26 (21 – 32) | 0.001 |
| ALBI score | -2.97 (-3.19 to -2.58) | -3.18 (-3.34 to -3.03) | <0.001 |
| HBV DNA (log IU/mL) | 1.3 (1.3 – 1.3) | 1.3 (1.3 – 2.7) | <0.001 |
| HBV DNA positivity (>20 IU/mL) (Yes) | 7/48 (14.6%) | 1098/2355 (46.6%) | <0.001 |
| HBeAg positivity (yes) | 4 (8.5%) | 226 (10.5%) | 0.444 |
| On nucleos(t)ide analogue therapy (yes) | 44 (91.7%) | 1328 (56.4%) | <0.001 |
| Controlled attenuation parameter (dB/m) | 216 (197 – 248) | 246 (206 – 291) | 0.001 |
| Liver stiffness (kPa) | 9.3 (7.5 – 16.3) | 5.5 (4.4 – 7.7) | <0.001 |
| ALBI: Albumin-Bilirubin, HBeAg: hepatitis B e antigen, HBV: hepatitis B virus | | | |

Supplementary table 3. Sensitivity analysis of multivariate regression on risk of HCC in all patients

|  |  |  |  |
| --- | --- | --- | --- |
|  | Odds ratio | 95% confidence interval | P value |
| Mild steatosis vs. no steatosis (n=2403) | 0.433 | 0.215 – 0.87 | 0.019 |
| Moderate steatosis vs. no or mild steatosis (n=2403) | 0.441 | 0.198 – 0.984 | 0.045 |
| Severe steatosis vs. no or mild or moderate steatosis (n=2403) | 0.333 | 0.126 – 0.883 | 0.027 |
| CAP when only patients with F3/F4 were included (n=371) | 0.996 | 0.987 – 1.005 | 0.339 |
| CAP when only patients without F3/F4 were included (n=2032) | 0.991 | 0.983 – 0.999 | 0.038 |
| CAP: controlled attenuation parameter, F3/F4: advanced fibrosis/ cirrhosis | | | |

Supplementary table 4. Univariate analysis of risk factors for HCC development in NA-treated patients (N=1349)

|  |  |  |  |
| --- | --- | --- | --- |
|  | HCC (n=44) | No HCC (n=1305) | P value |
| Age | 62.9 | 55.9 | <0.001 |
| Gender (male) | 33 (75%) | 837 (64.2%) | 0.153 |
| Body height (cm) | 164 | 164 | 0.953 |
| Body weight (kg) | 64.8 | 64.0 | 0.818 |
| Body mass index (kg/m2) | 23.6 | 23.4 | 0.734 |
| Waist circumference (cm) | 87 | 86 | 0.353 |
| Hip circumference (cm) | 96 | 96 | 0.915 |
| Systolic blood pressure (mmHg) | 140 | 134 | 0.49 |
| Diastolic blood pressure (mmHg) | 80 | 80 | 0.672 |
| Diabetes mellitus (yes) | 12/43 (27.9%) | 381/1223 (31.2%) | 0.739 |
| Glycated hemoglobin (%) | 5.7 | 5.6 | 0.921 |
| Dyslipidaemia (yes) | 20/44 (45.5%) | 706/1296 (54.5%) | 0.282 |
| Platelet count (x100/L) | 119 | 192 | <0.001 |
| Albumin (gram/L) | 43 | 45 | <0.001 |
| Bilirubin (umol/L) | 12 | 10 | 0.015 |
| Alanine aminotransferase (U/L) | 28 | 26 | 0.174 |
| Aspartate aminotransferase (U/L) | 30 | 26 | 0.001 |
| ALBI score | -2.93 (-3.17 to -2.55) | -3.19 (-3.34 to -3.01) | <0.001 |
| HBV DNA (log IU/mL) | 1.3 | 1.3 | 0.41 |
| HBV DNA positivity (>20 IU/mL) (Yes) | 3/44 (6.8%) | 191/1305 (14.6%) | 0.191 |
| HBeAg positivity (yes) | 4 (9.3%) | 168 (13.8%) | 0.28 |
| ETV (instead of other NA) | 81.6% | 85.9% | 0.289 |
| NA duration before transient elastography (months) | 67.7 | 77.0 | 0.38 |
| Controlled attenuation parameter (dB/m) | 214 | 241 | 0.002 |
| Liver stiffness (kPa) | 9.9 | 5.9 | <0.001 |
| ALBI: Albumin-Bilirubin, ETV: entecavir, HBeAg: hepatitis B virus, HBeAg: hepatitis e antigen, NA: nucleos(t)ide analogue | | | |

Supplementary table 5. Multivariate Cox regression analysis of risk factors for HCC development NA-treated patients

|  |  |  |  |
| --- | --- | --- | --- |
|  | Hazard ratio | 95% confidence interval | P value |
| Age (per year) | 1.059 | 1.029 – 1.09 | <0.001 |
| Platelet count (per 1 x 109/L) | 0.997 | 0.992 – 1.003 | 0.302 |
| Aspartate aminotransferase (per U/L) | 1.008 | 0.995 – 1.02 | 0.246 |
| ALBI score | 2.91 | 1.425 – 5.942 | 0.003 |
| Controlled attenuation parameter (per dB/m) | 0.993 | 0.987 – 0.999 | 0.015 |
| Liver stiffness (per kPa) | 1.014 | 0.987 – 1.042 | 0.323 |

ALBI: Albumin-Bilirubin

Associated univariate analysis presented in Supplementary Table 4.

(Albumin and bilirubin were incorporated in ALBI score)

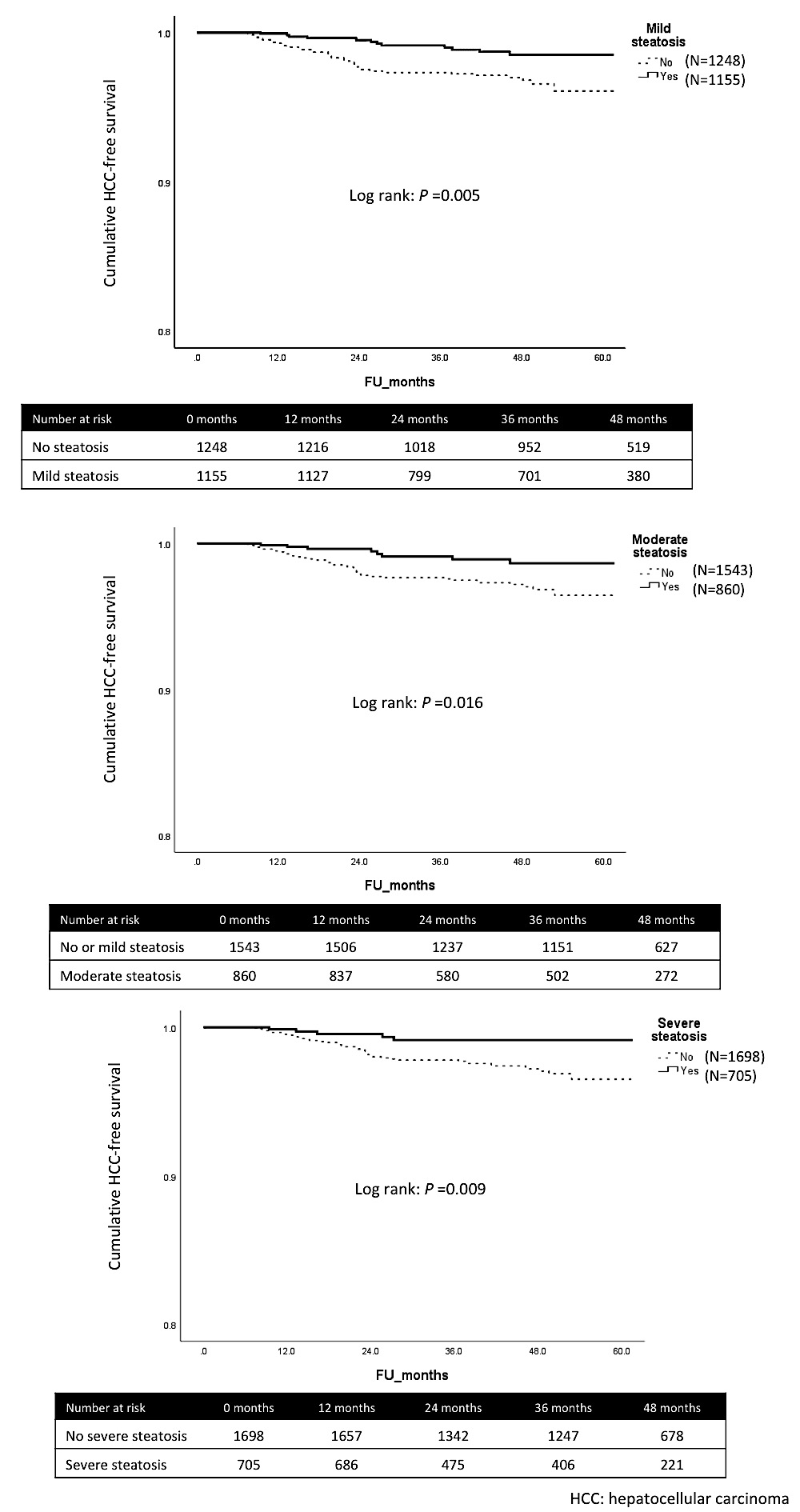
Supplementary table 6. Sensitivity analysis of multivariate regression on risk of HCC in NA-treated patients

|  |  |  |  |
| --- | --- | --- | --- |
|  | Odds ratio | 95% confidence interval | P value |
| Mild steatosis vs. no steatosis (n=1349) | 0.463 | 0.224 – 0.956 | 0.037 |
| Moderate steatosis vs. no or mild steatosis (n=1349) | 0.447 | 0.192 – 1.04 | 0.062 |
| Severe steatosis vs. no or mild or moderate steatosis (n=1349) | 0.315 | 0.109 – 0.912 | 0.033 |
| CAP when only patients with F3/F4 were included (n=275) | 0.995 | 0.987 – 1.004 | 0.312 |
| CAP when only patients without F3/F4 were included (n=1074) | 0.987 | 0.978 – 0.996 | 0.006 |
| CAP when only patients on ETV were included (n=1033)\* | 0.993 | 0.986 – 1.001 | 0.076 |
| CAP when only patients on TDF were included (n=171)\* | 0.998 | 0.983 – 1.014 | 0.83 |
| CAP when only patients on NA for ≥3 years were included (n=1013) | 0.992 | 0.985 – 1.000 | 0.037 |
| CAP when only patients on NA for <3 years were included (n=336) | 0.994 | 0.983 – 1.006 | 0.359 |
| \*145 patients were on other NAs (adefovir disoproxil fumarate, lamivudine, telbivudine or combination regimens)  CAP: controlled attenuation parameter, ETV: entecavir, F3/F4: advanced fibrosis/ cirrhosis, NA: nucleos(t)ide analogue, TDF: tenofovir disoproxil fumarate | | | |

Supplementary table 7. Baseline characteristics after propensity score matching\*

|  |  |  |  |
| --- | --- | --- | --- |
|  | Presence of hepatic steatosis† (N=957) | Absence of hepatic steatosis† (N=957) | ASD‡ |
| Age | 56.2 (48.5-63.2) | 55.6 (47.0-62.8) | 0.037 |
| Gender (male) | 563 (59%) | 559 (58%) | 0.004 |
| Liver stiffness (kPa) | 6 (4.7-8.4) | 5.3 (4.2-7.0) | 0.085 |
| Platelet | 210 (172-248) | 208 (167-249) | 0.055 |
| HBV DNA | 1.3 (1.3-2.7) | 1.3 (1.3-2.8) | 0.023 |
| albumin | 45 (43-47) | 45 (43-47) | 0.017 |
| Bilirubin | 10 (7-13) | 10 (8-13) | 0.009 |
| AST | 26 (21-33) | 25 (22-31) | 0.027 |
| On nucleos(t)ide analogue therapy (yes) | 544 (57%) | 537 (56%) | 0.007 |
| \* Matched by age, gender, liver stiffness, platelet, HBV DNA, albumin, bilirubin, AST and antiviral treatment.  † High CAP: CAP ≥ 248 dB/m; Low CAP: CAP <248 dB/m  ‡ ASD < 0.1 denotes variables were well balanced. | | | |

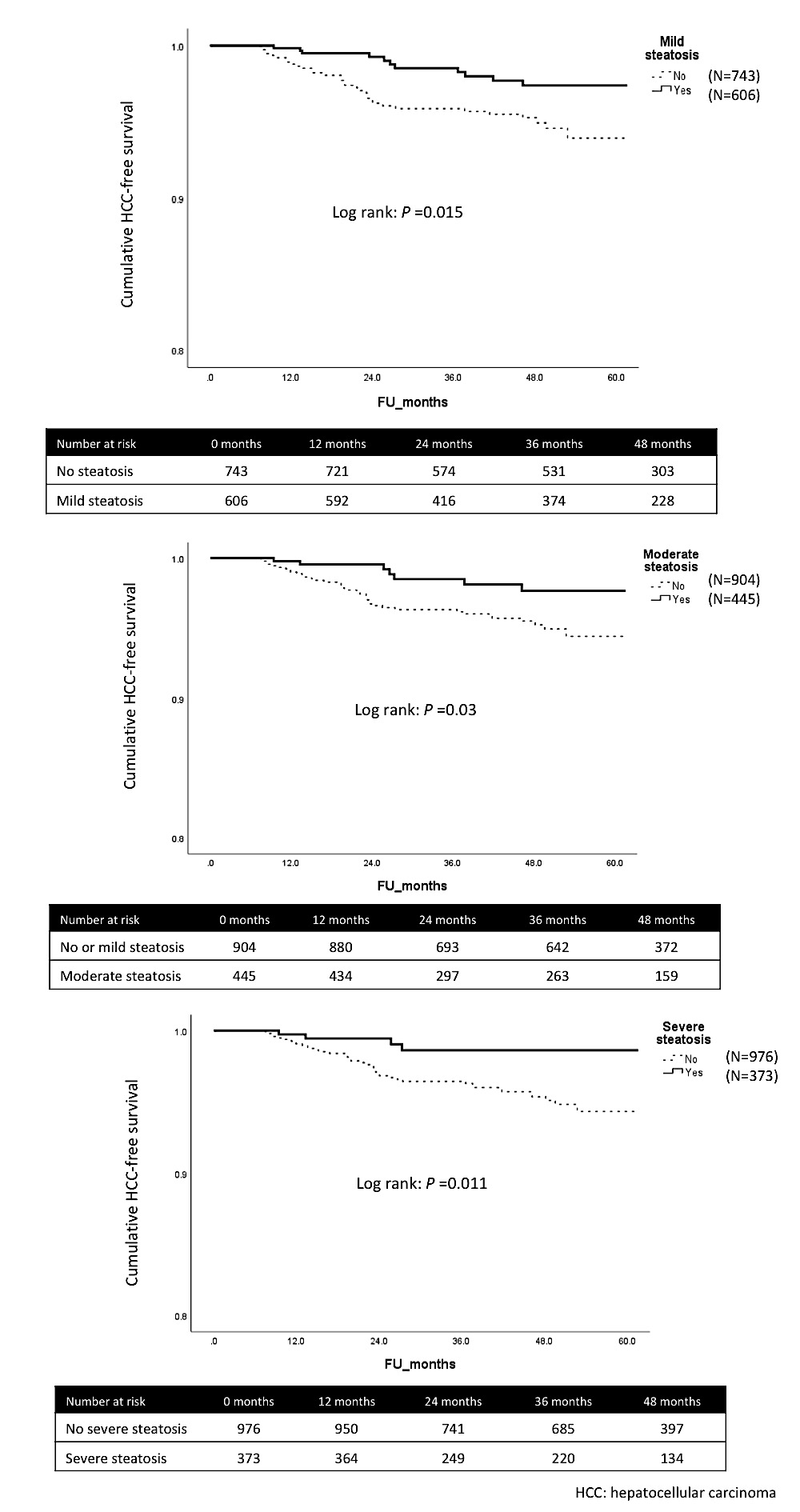
Supplementary figure 1. Cumulative HCC-free survival stratified by different degrees of hepatic steatosis in all patients



Supplementary figure 2. Cumulative HCC-free survival stratified by the severity of hepatic steatosis in NA-treated patients



Supplementary figure 3. Cumulative HCC-free survival stratified by different degrees of hepatic steatosis in NA-treated patients



Supplementary Figure 4. Potential link between hepatic fat, fibrosis and hepatocellular carcinoma

