

SUPPLEMENTARY DATA

Serum bioavailable, rather than total, 25-hydroxyvitamin D levels are associated with hepatocellular carcinoma survival

Ai-Ping Fang, Jing-An Long, Yao-Jun Zhang, Zhao-Yan Liu, Qi-Jiong Li, Dao-Ming Zhang, Yun Luo, Rong-Huan Zhong, Zhong-Guo Zhou, Yan-Jun Xu, Xiao-Jun Xu, Wen-Hua Ling, Min-Shan Chen, Hui-Lian Zhu

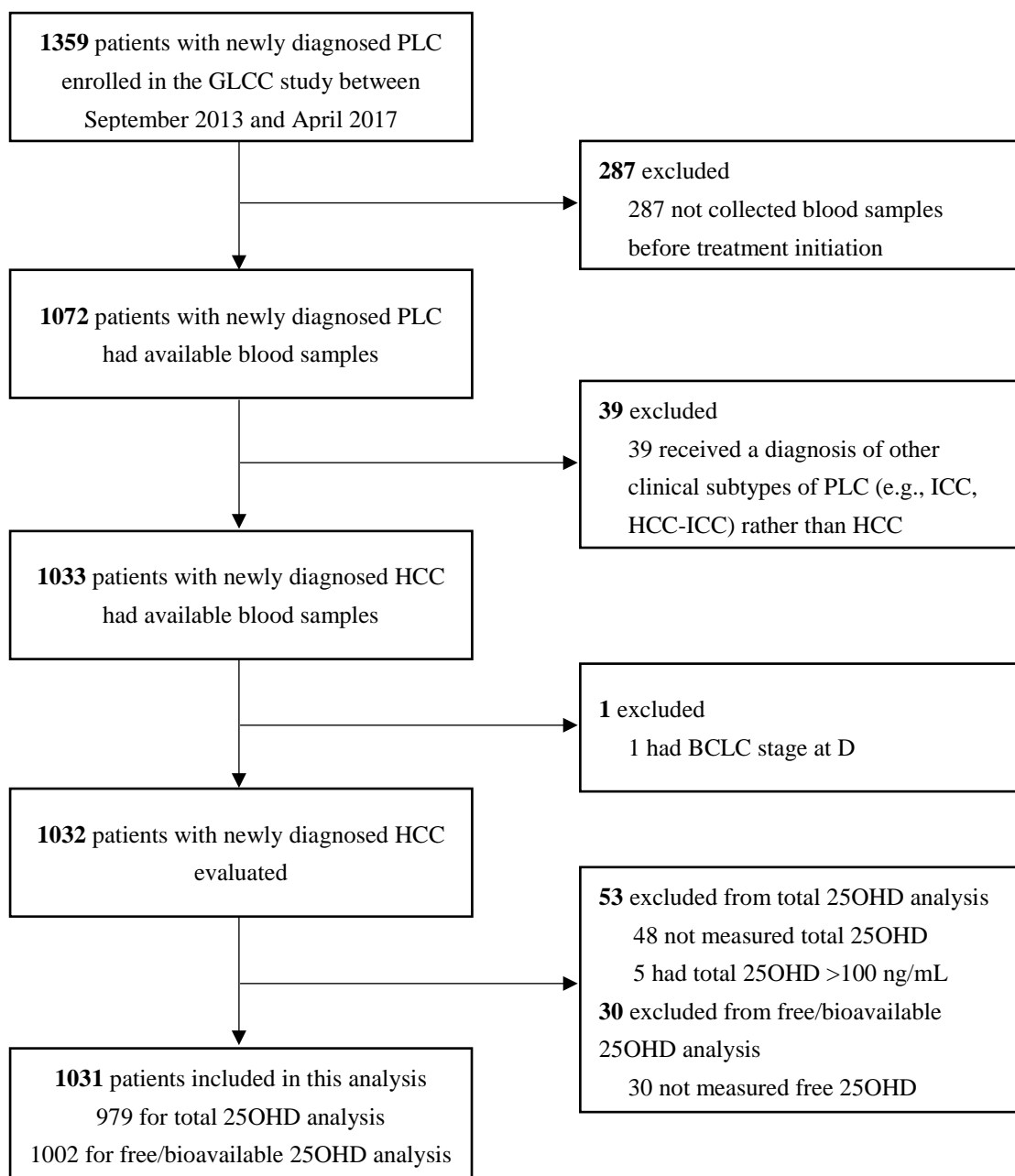
List of Supplementary Materials

Supplementary Fig. 1. The flowchart of participant selection for the present analysis in the Guangdong Liver Cancer Cohort study.

Supplementary Fig. 2. Correlations between serum 25-hydroxyvitamin D (25OHD) fractions.

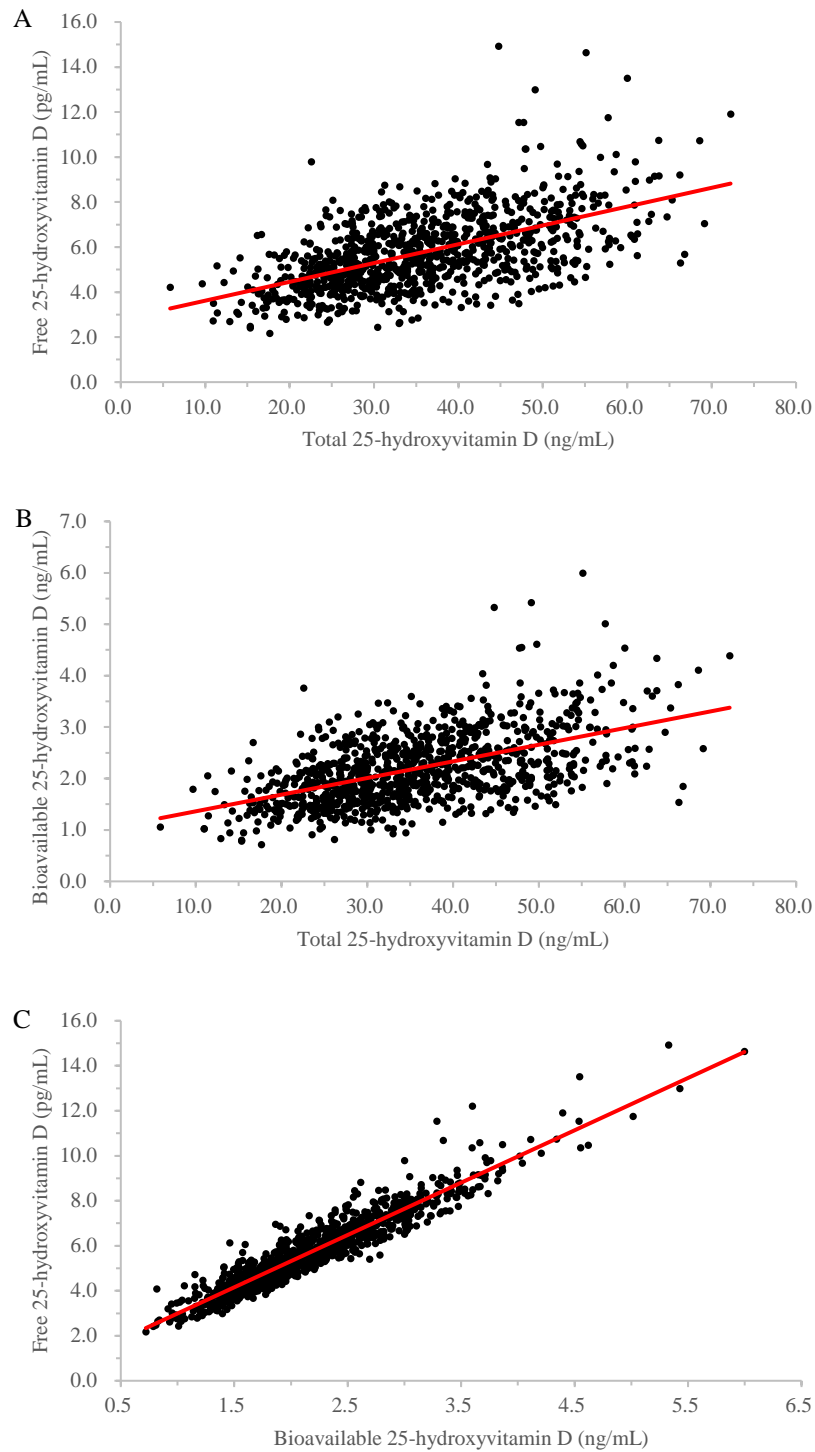
Supplementary Table 1. Baseline characteristics of participants by quartiles of serum bioavailable 25-hydroxyvitamin D (25OHD) levels in the Guangdong Liver Cancer Cohort study.

SUPPLEMENTARY FIGURES



Supplementary Figure 1. The flowchart of participant selection for the present analysis in the Guangdong Liver Cancer Cohort study.

Abbreviations: GLCC, Guangdong Liver Cancer Cohort; PLC, primary liver cancer; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; BCLC, Barcelona Clinic Liver Cancer.



Supplementary Figure 2. Correlations between serum 25-hydroxyvitamin D (25OHD) fractions. A. Correlation between free 25OHD and total 25OHD. B. Correlation between bioavailable 25OHD and total 25OHD. C. Correlation between free 25OHD and bioavailable 25OHD.

SUPPLEMENTARY TABLES

Supplementary Table 1. Baseline characteristics of participants by quartiles of serum bioavailable 25-hydroxyvitamin D (25OHD) levels in the Guangdong Liver Cancer Cohort study

| | Quartile of bioavailable 25OHD | | | | <i>P</i> value |
|---|--------------------------------|-------------------|-------------------|-------------------|----------------|
| | Q1 (n=250) | Q2 (n=251) | Q3 (n=251) | Q4 (n=250) | |
| Age at diagnosis (years) | 53.8±12.6 | 53.6±12.5 | 52.8±11.2 | 52.0±11.6 | 0.330 |
| Sex, No. (%) | | | | | 0.088 |
| Women | 36 (14.4) | 33 (13.1) | 33 (13.1) | 19 (7.6) | |
| Men | 214 (85.6) | 218 (86.9) | 218 (86.9) | 231 (92.4) | |
| BMI at diagnosis (kg/m ²) | 22.7±3.1 | 22.8±3.4 | 22.6±3.0 | 23.0±3.3 | 0.580 |
| Education level, No. (%) | | | | | 0.969 |
| Primary school or below | 49 (19.8) | 50 (19.9) | 53 (21.2) | 53 (21.3) | |
| Secondary school | 146 (58.9) | 151 (60.2) | 146 (58.4) | 152 (61.0) | |
| Collage or higher | 53 (21.4) | 50 (19.9) | 51 (20.4) | 44 (17.7) | |
| Residence, No. (%) | | | | | 0.226 |
| Urban | 184 (73.6) | 177 (70.5) | 181 (72.1) | 164 (65.6) | |
| Rural | 66 (26.4) | 74 (29.5) | 70 (27.9) | 86 (34.4) | |
| Physical activity (MET·hour/day) ^a | 19.3 (15.8, 26.2) | 20.5 (16.3, 28.3) | 20.9 (16.3, 28.4) | 21.7 (17.5, 30.2) | 0.015 |
| Smoking status, No. (%) | | | | | 0.244 |
| Never smoker | 95 (38.0) | 110 (43.8) | 113 (45.0) | 109 (43.6) | |
| Former smoker | 76 (30.4) | 75 (29.9) | 63 (25.1) | 57 (22.8) | |
| Current smoker | 79 (31.6) | 66 (26.3) | 75 (29.9) | 84 (33.6) | |
| Alcohol drinking status, No. (%) | | | | | 0.830 |
| Never drinker | 147 (58.8) | 151 (60.2) | 138 (55.0) | 149 (59.6) | |
| Former drinker | 44 (17.6) | 36 (14.3) | 42 (16.7) | 38 (15.2) | |

| | Quartile of bioavailable 25OHD | | | | <i>P</i> value |
|--------------------------------|--------------------------------|--------------------|--------------------|--------------------|----------------|
| | Q1 (n=250) | Q2 (n=251) | Q3 (n=251) | Q4 (n=250) | |
| Current drinker | 59 (23.6) | 64 (25.5) | 71 (28.3) | 63 (25.2) | |
| Family history of PLC, No. (%) | | | | | 0.919 |
| No | 214 (85.6) | 216 (86.1) | 218 (86.9) | 219 (87.6) | |
| Yes | 36 (14.4) | 35 (13.9) | 33 (13.1) | 31 (12.4) | |
| HBV/HCV infection, No. (%) | | | | | 0.320 |
| HBsAg (-) and anti-HCV (-) | 22 (8.8) | 29 (11.6) | 17 (6.8) | 23 (9.2) | |
| HBsAg (+) and/or anti-HCV (+) | 228 (91.2) | 222 (88.4) | 234 (93.2) | 227 (90.8) | |
| Fatty liver disease, No. (%) | | | | | 0.127 |
| No | 217 (86.8) | 202 (80.5) | 202 (80.5) | 213 (85.2) | |
| Yes | 33 (13.2) | 49 (19.5) | 49 (19.5) | 37 (14.8) | |
| Liver cirrhosis, No. (%) | | | | | 0.291 |
| No | 83 (33.2) | 83 (33.1) | 96 (38.2) | 99 (39.6) | |
| Yes | 167 (66.8) | 168 (66.9) | 155 (61.8) | 151 (60.4) | |
| Serum ALT (U/L) | 39.2 (27.1, 57.9) | 36.6 (25.0, 60.0) | 38.1 (26.0, 57.3) | 41.6 (28.4, 59.9) | 0.284 |
| Serum AST (U/L) | 45.4 (28.8, 73.3) | 39.2 (27.7, 67.0) | 38.6 (28.2, 58.8) | 39.0 (27.3, 60.5) | 0.056 |
| Serum GGT (U/L) | 90.2 (46.1, 178.4) | 71.6 (41.3, 154.9) | 74.2 (39.3, 140.1) | 64.6 (36.0, 134.6) | 0.016 |
| Serum ALP (U/L) | 107.4 (80.1, 157.0) | 98.1 (77.9, 125.6) | 95.9 (74.9, 126.1) | 95.1 (77.6, 124.1) | 0.009 |
| Serum bilirubin (mmol/L) | 14.1 (10.9, 19.6) | 13.9 (10.7, 19.3) | 13.5 (10.1, 17.8) | 14.0 (10.5, 18.0) | 0.197 |
| Serum albumin (g/L) | 39.7±4.8 | 41.8±3.8 | 42.4±3.5 | 43.9±3.6 | <0.001 |
| Child-Pugh score, No. (%) | | | | | <0.001 |
| A | 236 (94.4) | 249 (99.2) | 251 (100.0) | 248 (99.2) | |
| B | 14 (5.6) | 2 (0.8) | 0 (0.0) | 2 (0.8) | |
| eGFR, No. (%) | | | | | 0.660 |

| | Quartile of bioavailable 25OHD | | | | <i>P</i> value |
|-------------------------------------|--------------------------------|------------|------------|------------|----------------|
| | Q1 (n=250) | Q2 (n=251) | Q3 (n=251) | Q4 (n=250) | |
| ≥60 mL/min per 1.73 m ² | 241 (96.4) | 243 (96.8) | 242 (96.4) | 237 (94.8) | |
| <60 mL/min per 1.73 m ² | 9 (3.6) | 8 (3.2) | 9 (3.6) | 13 (5.2) | |
| Serum CRP, No. (%) | | | | | <0.001 |
| ≤3.0 mg/L | 116 (46.4) | 124 (49.4) | 127 (50.6) | 163 (65.5) | |
| >3.0 mg/L | 134 (53.6) | 127 (50.6) | 124 (49.4) | 86 (34.5) | |
| Serum AFP, No. (%) | | | | | 0.270 |
| ≤400 ng/mL | 152 (60.8) | 153 (61.0) | 141 (56.2) | 162 (64.8) | |
| >400 ng/mL | 98 (39.2) | 98 (39.0) | 110 (43.8) | 88 (35.2) | |
| BCLC stage, No. (%) | | | | | 0.070 |
| 0 | 21 (8.4) | 29 (11.6) | 23 (9.2) | 37 (14.8) | |
| A | 94 (37.6) | 66 (26.3) | 83 (33.1) | 74 (29.6) | |
| B | 24 (9.6) | 39 (15.5) | 27 (10.8) | 30 (12.0) | |
| C | 111 (44.4) | 117 (46.6) | 118 (47.0) | 109 (43.6) | |
| Cancer treatment, No. (%) | | | | | 0.004 |
| Hepatectomy/liver transplantation | 86 (34.4) | 112 (44.6) | 124 (49.4) | 130 (52.0) | |
| Local ablation | 34 (13.6) | 28 (11.2) | 21 (8.4) | 30 (12.0) | |
| Hepatic arterial intervention | 114 (45.6) | 105 (41.8) | 94 (37.5) | 81 (32.4) | |
| Other treatments ^b | 16 (6.4) | 6 (2.4) | 12 (4.8) | 9 (3.6) | |
| Season of blood collection, No. (%) | | | | | 0.004 |
| Spring (Feb-Mar) | 50 (20.0) | 48 (19.1) | 50 (19.9) | 21 (8.4) | |
| Summer (Apr-Sep) | 120 (48.0) | 109 (43.4) | 104 (41.4) | 133 (53.2) | |
| Autumn (Oct-Nov) | 48 (19.2) | 54 (21.5) | 59 (23.5) | 47 (18.8) | |
| Winter (Dec-Jan) | 32 (12.8) | 40 (15.9) | 38 (15.1) | 49 (19.6) | |

| | Quartile of bioavailable 25OHD | | | | <i>P</i> value |
|----------------------------|--------------------------------|-------------|------------|-------------|----------------|
| | Q1 (n=250) | Q2 (n=251) | Q3 (n=251) | Q4 (n=250) | |
| Serum calcium (mmol/L) | 2.25±0.13 | 2.28±0.11 | 2.28±0.12 | 2.32±0.12 | <0.001 |
| Total 25OHD (ng/mL) | 28.45±9.00 | 34.73±14.72 | 37.88±9.73 | 43.90±13.28 | <0.001 |
| Free 25OHD (pg/mL) | 4.01±0.70 | 5.10±0.58 | 6.12±0.63 | 7.86±1.42 | <0.001 |
| Bioavailable 25OHD (ng/mL) | 1.43±0.22 | 1.92±0.11 | 2.33±0.13 | 3.10±0.51 | <0.001 |

Abbreviations: 25OHD, 25-hydroxyvitamin D; BMI, body mass index; PLC, primary liver cancer; HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; anti-HCV, hepatitis C antibody; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer.

^a Including occupation, transportation, housework, and leisure-time physical activity.

^b Including radiation therapy and systemic treatment (e.g., molecular targeted therapy, systemic chemotherapy, traditional Chinese medication).