Supplementary Materials
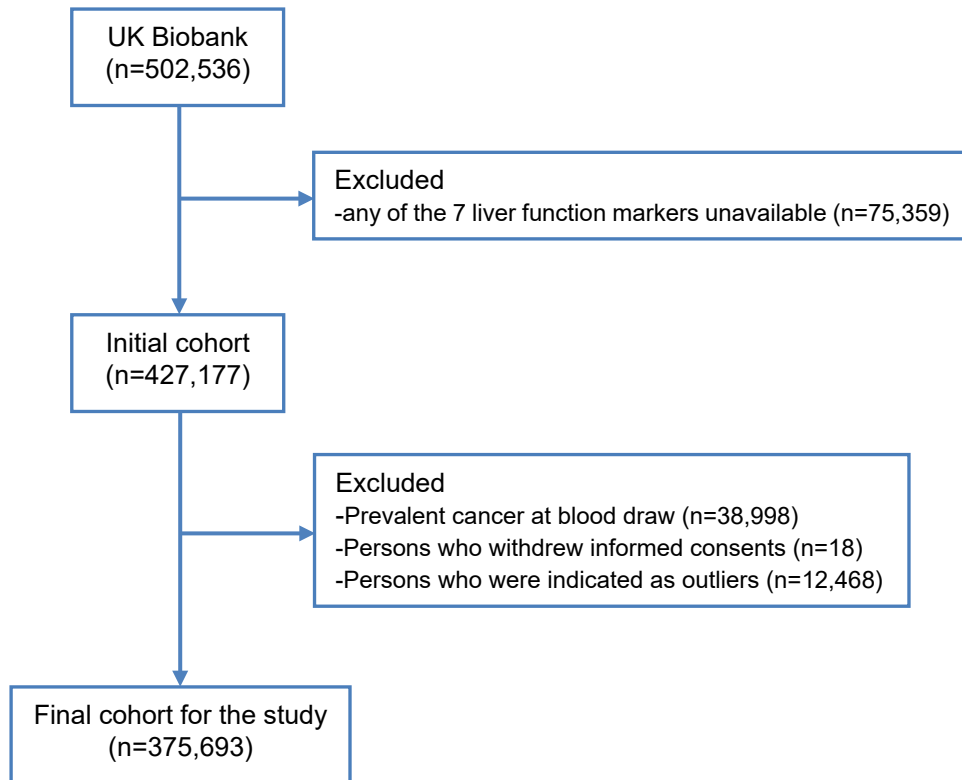
Circulating Liver Function Markers and Colorectal Cancer Risk: A Prospective Cohort Study in the UK Biobank

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Supplementary Figures

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┌─────────────────────┐
│ UK Biobank          │
│ (n=502,536)         │
└─────────────────────┘
          │
          │        ┌──────────────────────────────────────────────────────┐
          ├───────▶│ Excluded                                             │
          │        │ -any of the 7 liver function markers unavailable      │
          │        │  (n=75,359)                                          │
          │        └──────────────────────────────────────────────────────┘
          ▼
┌─────────────────────┐
│ Initial cohort      │
│ (n=427,177)         │
└─────────────────────┘
          │
          │        ┌──────────────────────────────────────────────────────┐
          ├───────▶│ Excluded                                             │
          │        │ -Prevalent cancer at blood draw (n=38,998)           │
          │        │ -Persons who withdrew informed consents (n=18)        │
          │        │ -Persons who were indicated as outliers (n=12,468)    │
          │        └──────────────────────────────────────────────────────┘
          ▼
┌─────────────────────┐
│ Final cohort for    │
│ the study           │
│ (n=375,693)         │
└─────────────────────┘
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Supplementary Figure 1. Flow chart of participant selection in the UK Biobank

Supplementary Tables

Supplementary Table 1. Normal range for circulating liver function markers and the percentage of our study participants within the normal range [a]

Biomarker	Normal range	Percentage of participants within the normal range
Alanine transaminase (ALT)	7-52 U/L	97%
Aspartate transaminase (AST)	13-39 U/L	96%
Total bilirubin (TBIL)	5-17 μmol/L	89%
Gamma glutamyltransferase (GGT)	9-64 U/L	91%
Alkaline phosphatase (ALP)	34-104 U/L	85%
Total Protein (TP)	64-89 g/L	99%
Albumin (ALB)	35-57 g/L	100%

[a] Normal range is based on Beckman Coulter AU5800 determined reference range for circulating liver function markers (https://www.beckmancoulter.com/support/tech-docs).

Supplementary Table 2. Intraclass correlation coefficients of circulating liver function markers measured in repeated samples [a]

	Intraclass correlation coefficient (95% confidence interval)
Alanine transaminase (ALT)	0.51 (0.49-0.52)
Aspartate transaminase (AST)	0.49 (0.48-0.51)
Total bilirubin (TBIL)	0.74 (0.73-0.75)
Gamma glutamyltransferase (GGT)	0.62 (0.60-0.63)
Alkaline phosphatase (ALP)	0.75 (0.74-0.76)
Total Protein (TP)	0.50 (0.48-0.51)
Albumin (ALB)	0.48 (0.47-0.50)

[a] Intraclass correlation coefficients were calculated in the subsample of participants with repeat measurements of circulating liver function markers after excluding the cancer patients diagnosed between two repeat measurements (n=11320).

Supplementary Table 3. Hazard ratios (95% confidence intervals) of early-, mid-, and late-onset colorectal cancer associated with circulating levels of liver function markers [a]

	Early-onset CRC (age<50 years) (n=93)	Mid-onset CRC (50≤age<60 years) (n=509)	Late-onset CRC (age≥60 years) (n=2060)	*P* for heterogeneity [b]
Alanine transaminase (ALT)				
HR (95% CI), decile 10 vs. 1	0.65 (0.28-1.54)	0.68 (0.45-1.02)	0.63 (0.50-0.79)	0.94
HR (95% CI), per 1-SD increment	0.96 (0.77-1.21)	0.91 (0.83-1.00)	0.88 (0.83-0.92)	0.60
Aspartate transaminase (AST)				
HR (95% CI), decile 10 vs. 1	1.02 (0.45-2.31)	0.68 (0.46-1.01)	0.62 (0.50-0.77)	0.48
HR (95% CI), per 1-SD increment	0.96 (0.76-1.20)	0.94 (0.85-1.03)	0.91 (0.87-0.96)	0.81
Total bilirubin (TBIL)				
HR (95% CI), decile 10 vs. 1	0.57 (0.23-1.41)	1.16 (0.80-1.70)	0.79 (0.65-0.97)	0.15
HR (95% CI), per 1-SD increment	0.88 (0.70-1.11)	1.05 (0.96-1.14)	0.96 (0.92-1.01)	0.18
Gamma glutamyltransferase (GGT)				
HR (95% CI), decile 10 vs. 1	1.23 (0.43-3.50)	0.60 (0.40-0.90)	0.78 (0.62-0.97)	0.35
HR (95% CI), per 1-SD increment	0.99 (0.77-1.25)	0.95 (0.86-1.04)	0.99 (0.94-1.03)	0.77
Alkaline phosphatase (ALP)				
HR (95% CI), decile 10 vs. 1	3.42 (1.28-9.10)	1.03 (0.71-1.50)	1.02 (0.84-1.25)	0.06
HR (95% CI), per 1-SD increment	1.11 (0.88-1.40)	1.07 (0.97-1.17)	1.01 (0.96-1.05)	0.40
Total Protein (TP)				
HR (95% CI), decile 10 vs. 1	0.31 (0.12-0.79)	0.72 (0.48-1.08)	0.73 (0.60-0.89)	0.21
HR (95% CI), per 1-SD increment	0.78 (0.63-0.97)	0.95 (0.86-1.04)	0.91 (0.87-0.95)	0.26
Albumin (ALB)				
HR (95% CI), decile 10 vs. 1	0.54 (0.22-1.33)	0.57 (0.37-0.87)	0.71 (0.58-0.88)	0.57
HR (95% CI), per 1-SD increment	0.89 (0.72-1.10)	0.87 (0.80-0.96)	0.92 (0.88-0.96)	0.65

Abbreviations: CI=Confidence interval; CRC=Colorectal cancer; HR=Hazard ratio; SD=Standard deviation.

[a] Multivariable Cox regression model with age as the underlying time scale was used and adjusted for sex, race (white, non-white, unknown), fasting status, age at recruitment, Townsend deprivation index (continuous), waist circumference / hip circumference (continuous), height (continuous), BMI (continuous), C-reactive protein (continuous), total physical activity (quintile), alcohol status and consumption frequency (never, former, current- special occasions only, current- 1-3 times per month, current- 1-2 times per week, current- 3-4 times per week, current- daily/almost daily, unknown), smoking status and intensity (never, former, current- <15 per day, current- ≥15 per day, current- intensity unknown, unknown), frequency of red and processed meat consumption (never, <1, =1, 2-4, 5-6, ≥7 occasions per week, unknown), frequency of oily fish consumption (never, <1, =1, 2-4, 5-6, ≥7 occasions per week, unknown), family history of cancer (no, yes, unknown), educational level (college/university degree, non-college/university degree, unknown), regular aspirin use (no, yes, unknown), bowel cancer screening (no, yes, unknown), and overall health ranking (excellent, good, fair, poor, unknown).

[b] P for heterogeneity was calculated using the contrast method using the contrast method based on a fully unconstrained approach.

Supplementary Table 4. Stratified analyses of the association between circulating levels of liver function markers and colorectal cancer risk in the UK Biobank [a]

	No. of CRC cases	ALT	AST	TBIL	GGT	ALP	TP	ALB
Age at recruitment								
<60	917	0.89 (0.83-0.96)	0.91 (0.84-0.97)	1.04 (0.98-1.11)	0.96 (0.89-1.03)	1.06 (0.99-1.14)	0.94 (0.88-1.00)	0.90 (0.84-0.96)
≥60	1745	0.88 (0.83-0.93)	0.92 (0.88-0.97)	0.94 (0.89-0.99)	0.99 (0.94-1.04)	0.99 (0.95-1.05)	0.89 (0.85-0.94)	0.91 (0.87-0.96)
P for interaction [b]		0.73	0.66	0.10	0.26	0.04	0.14	0.78
Sex								
Women	1127	0.86 (0.79-0.93)	0.93 (0.87-0.99)	0.97 (0.90-1.04)	0.96 (0.89-1.03)	1.00 (0.94-1.06)	0.92 (0.86-0.97)	0.88 (0.82-0.93)
Men	1535	0.89 (0.85-0.95)	0.91 (0.86-0.96)	0.99 (0.94-1.04)	0.98 (0.93-1.03)	1.04 (0.98-1.09)	0.90 (0.86-0.95)	0.93 (0.88-0.98)
P for interaction [b]		0.07	0.88	0.81	0.10	0.17	0.60	0.31
Smoking status								
Never	1232	0.89 (0.83-0.96)	0.92 (0.87-0.98)	0.98 (0.92-1.03)	0.96 (0.90-1.02)	1.03 (0.97-1.09)	0.88 (0.83-0.93)	0.86 (0.81-0.91)
Former	1150	0.86 (0.80-0.92)	0.90 (0.84-0.96)	0.97 (0.91-1.03)	0.99 (0.93-1.05)	1.00 (0.94-1.06)	0.93 (0.87-0.98)	0.92 (0.86-0.98)
Current	264	0.97 (0.84-1.12)	0.99 (0.88-1.13)	1.15 (1.01-1.31)	0.97 (0.86-1.10)	1.01 (0.89-1.14)	1.00 (0.88-1.13)	1.10 (0.97-1.25)
P for interaction [b]		0.57	0.42	0.10	0.32	0.30	0.17	0.01
Alcohol consumption								
None	185	0.86 (0.71-1.03)	0.75 (0.63-0.90)	0.98 (0.83-1.15)	0.96 (0.80-1.13)	1.06 (0.92-1.21)	0.92 (0.80-1.07)	0.89 (0.77-1.04)
Special occasions only	269	0.84 (0.72-0.98)	0.89 (0.78-1.01)	0.94 (0.82-1.09)	1.03 (0.90-1.17)	0.92 (0.82-1.04)	0.88 (0.78-1.00)	0.90 (0.79-1.01)
1-3 times per month	244	0.96 (0.83-1.11)	0.99 (0.86-1.13)	1.09 (0.96-1.23)	1.09 (0.96-1.25)	1.06 (0.93-1.20)	0.94 (0.83-1.07)	1.01 (0.89-1.15)
1-2 times per week	663	0.84 (0.77-0.93)	0.90 (0.83-0.98)	0.95 (0.87-1.03)	0.91 (0.83-1.00)	0.98 (0.90-1.06)	0.93 (0.86-1.01)	0.89 (0.82-0.97)
3-4 times per week	617	0.89 (0.81-0.98)	0.90 (0.83-0.99)	0.97 (0.89-1.06)	0.98 (0.90-1.06)	1.08 (0.99-1.18)	0.88 (0.81-0.96)	0.88 (0.81-0.96)
Daily / almost daily	677	0.88 (0.81-0.97)	0.94 (0.87-1.02)	1.00 (0.92-1.08)	0.96 (0.89-1.03)	0.99 (0.91-1.08)	0.90 (0.83-0.97)	0.91 (0.84-0.98)
P for interaction [b]		0.80	0.19	0.84	0.64	0.55	0.95	0.79
Body mass index (kg/m^2)								
<25	719	0.82 (0.73-0.92)	0.90 (0.82-0.99)	0.99 (0.92-1.07)	0.94 (0.85-1.03)	1.03 (0.95-1.11)	0.92 (0.86-1.00)	0.85 (0.79-0.92)
≥25	1943	0.89 (0.85-0.93)	0.92 (0.87-0.96)	0.98 (0.93-1.02)	0.98 (0.94-1.03)	1.01 (0.96-1.06)	0.90 (0.86-0.94)	0.92 (0.88-0.97)
P for interaction [b]		0.10	0.41	0.88	0.22	0.51	0.44	0.10

Abbreviations: ALB=Albumin; ALP=Alkaline phosphatase; ALT=Alanine transaminase; AST=Aspartate transaminase; CRC=colorectal cancer; GGT=Gamma glutamyltransferase; TBIL=Total bilirubin; TP=Total Protein.

Supplementary Table 5. Sensitivity analyses of the association between circulating levels of liver function markers and colorectal cancer risk in the UK Biobank [a]

	No. of CRC cases	ALT	AST	TBIL	GGT	ALP	TP	ALB
The primary findings for the total cohort								
HR (95% CI), decile 10 vs. 1	2662	0.62 (0.51-0.75)	0.63 (0.53-0.75)	0.85 (0.72-1.02)	0.74 (0.61-0.89)	1.03 (0.87-1.23)	0.70 (0.59-0.84)	0.66 (0.55-0.79)
HR (95% CI) per 1-SD		0.88 (0.84-0.92)	0.91 (0.88-0.95)	0.98 (0.94-1.02)	0.98 (0.94-1.02)	1.02 (0.98-1.06)	0.91 (0.88-0.95)	0.90 (0.86-0.93)
Excluding the first two years of follow-up (n=1962)								
HR (95% CI), decile 10 vs. 1	2020	0.72 (0.58-0.90)	0.69 (0.56-0.85)	1.04 (0.84-1.28)	0.77 (0.62-0.96)	1.01 (0.82-1.23)	0.72 (0.59-0.89)	0.68 (0.55-0.84)
HR (95% CI) per 1-SD		0.91 (0.86-0.96)	0.93 (0.88-0.97)	1.01 (0.97-1.06)	0.99 (0.94-1.03)	1.01 (0.97-1.06)	0.92 (0.88-0.96)	0.93 (0.88-0.97)
Excluding participants with abnormally low or high levels of circulating liver function markers (n=44987)								
HR (95% CI), decile 10 vs. 1	2305	0.57 (0.45-0.72)	0.57 (0.46-0.71)	0.85 (0.70-1.03)	0.74 (0.59-0.94)	1.08 (0.88-1.32)	0.70 (0.58-0.86)	0.62 (0.50-0.75)
HR (95% CI) per 1-SD		0.83 (0.78-0.88)	0.85 (0.80-0.90)	0.97 (0.93-1.02)	0.92 (0.86-0.99)	1.01 (0.96-1.06)	0.90 (0.86-0.94)	0.89 (0.85-0.93)
Excluding participants with inflammatory bowel disease at recruitment (n=2480) [b]								
HR (95% CI), decile 10 vs. 1	2637	0.63 (0.52-0.76)	0.63 (0.52-0.75)	0.87 (0.73-1.04)	0.74 (0.61-0.90)	1.03 (0.87-1.23)	0.70 (0.59-0.84)	0.67 (0.55-0.80)
HR (95% CI) per 1-SD		0.88 (0.84-0.92)	0.91 (0.88-0.95)	0.98 (0.94-1.03)	0.98 (0.94-1.02)	1.01 (0.97-1.05)	0.91 (0.87-0.94)	0.91 (0.87-0.95)
Excluding participants with hepatitis and other liver/hepatobiliary disease at recruitment (n=8748) [b]								
HR (95% CI), decile 10 vs. 1	2604	0.61 (0.50-0.74)	0.62 (0.52-0.74)	0.85 (0.71-1.02)	0.73 (0.60-0.89)	1.02 (0.86-1.22)	0.70 (0.59-0.84)	0.65 (0.54-0.79)
HR (95% CI) per 1-SD		0.87 (0.83-0.92)	0.91 (0.87-0.95)	0.98 (0.94-1.02)	0.97 (0.93-1.01)	1.01 (0.97-1.05)	0.91 (0.87-0.95)	0.90 (0.87-0.94)
Excluding participants with cardiovascular disease at recruitment (n=42948) [b]								
HR (95% CI), decile 10 vs. 1	2254	0.61 (0.49-0.75)	0.63 (0.51-0.77)	0.85 (0.70-1.03)	0.75 (0.61-0.92)	1.15 (0.96-1.40)	0.72 (0.60-0.88)	0.65 (0.53-0.79)
HR (95% CI) per 1-SD		0.87 (0.83-0.92)	0.92 (0.88-0.96)	0.98 (0.93-1.02)	0.97 (0.93-1.02)	1.03 (0.99-1.08)	0.91 (0.87-0.95)	0.90 (0.86-0.94)
Excluding participants with diabetes at recruitment (n=7421) [b]								
HR (95% CI), decile 10 vs. 1	2584	0.61 (0.50-0.74)	0.62 (0.52-0.75)	0.84 (0.71-1.01)	0.73 (0.60-0.88)	1.06 (0.88-1.26)	0.71 (0.59-0.85)	0.65 (0.54-0.78)
HR (95% CI) per 1-SD		0.87 (0.83-0.92)	0.91 (0.87-0.95)	0.98 (0.94-1.02)	0.97 (0.93-1.01)	1.02 (0.98-1.06)	0.91 (0.87-0.94)	0.90 (0.86-0.94)
Adjustment for additional lifestyle factors for the total cohort [c]								

HR (95% CI), decile 10 vs. 1	2662	0.62 (0.51-0.75)	0.63 (0.52-0.75)	0.84 (0.71-1.01)	0.73 (0.61-0.89)	1.02 (0.86-1.21)	0.70 (0.59-0.84)	0.66 (0.55-0.79)
HR (95% CI) per 1-SD		0.88 (0.84-0.92)	0.91 (0.87-0.95)	0.98 (0.94-1.02)	0.97 (0.93-1.01)	1.01 (0.97-1.05)	0.91 (0.87-0.94)	0.90 (0.87-0.94)

Abbreviations: ALB=Albumin; ALP=Alkaline phosphatase; ALT=Alanine transaminase; AST=Aspartate transaminase; CI=Confidence interval; CRC=Colorectal cancer; GGT=Gamma glutamyltransferase; HR=Hazard ratio; SD=Standard deviation; TBIL=Total bilirubin; TP=Total protein.

[a] Multivariable Cox regression model with age as the underlying time scale was used and adjusted for sex, race (white, non-white, unknown), fasting status, age at recruitment, Townsend deprivation index (continuous), waist circumference / hip circumference (continuous), height (continuous), BMI (continuous), C-reactive protein (continuous), total physical activity (quintile), alcohol status and consumption frequency (never, former, current- special occasions only, current- 1-3 times per month, current- 1-2 times per week, current- 3-4 times per week, current- daily/almost daily, unknown), smoking status and intensity (never, former, current- <15 per day, current- ≥15 per day, current- intensity unknown, unknown), frequency of red and processed meat consumption (never, <1, =1, 2-4, 5-6, ≥7 occasions per week, unknown), frequency of oily fish consumption (never, <1, =1, 2-4, 5-6, ≥7 occasions per week, unknown), family history of cancer (no, yes, unknown), educational level (college/university degree, non-college/university degree, unknown), regular aspirin use (no, yes, unknown), bowel cancer screening (no, yes, unknown), and overall health ranking (excellent, good, fair, poor, unknown)).

[b] The diagnoses of inflammatory bowel disease, hepatitis and other liver disease and hepatobiliary disease, cardiovascular disease, and diabetes were all coded according to the 10th Revision of the International Classification of Diseases (ICD-10).

[c] Additional adjustment for raw vegetable intake (continuous), cooked vegetable intake (continuous), fresh fruit intake (continuous), dried fruit intake (continuous), frequency of poultry consumption (never, <1, =1, 2-4, 5-6, ≥7 occasions per week, unknown), coffee intake (continuous), vitamin supplements (any of vitamin A, vitamin B, vitamin C, vitamin D, vitamin E, folate, and multivitamins, or none), and mineral and other dietary supplements (any of calcium, zinc, iron, selenium, glucosamine, and fish oil, or none).