

Association between C-Reactive Protein, Incident Liver Cancer, and Chronic Liver Disease Mortality in the Linxian Nutrition Intervention Trials: A Nested Case–Control Study

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Abstract

Background: C-reactive protein (CRP) is a marker of systemic inflammation that has been associated with the incidence and prognosis for a number of different cancers. Recent data suggest that CRP may be a prognostic factor for liver cancer and cirrhosis. However, few long-term studies are available.

Methods: We prospectively examined associations between serum CRP and subsequent risk of liver cancer incidence or chronic liver disease mortality in a nested case–control study performed in the Linxian Nutrition Intervention Trials cohort. Baseline serum CRP was measured for 220 incident liver cancer cases, 276 participants who died of chronic liver disease, and 1,018 age-, sex-, and trial-matched controls. Unconditional logistical regression models were used to estimate ORs and 95% confidence intervals (CI).

Results: Compared with the lowest quartile, subjects in the fourth quartile of serum CRP had a higher risk of liver cancer incidence (OR, 1.63; 95% CI, 1.06–2.51), with a significant P_{trend} across quartiles ($P = 0.01$). The association with liver cancer was only significant among men (Q4 vs. Q1; OR, 2.00; 1.10–3.62), but not among women (Q4 vs. Q1; OR, 1.15; 0.60–2.22). For chronic liver disease deaths, the corresponding risk estimate in men and women was 2.95 (1.90–4.57), with a monotonic trend ($P < 0.001$).

Conclusions: Higher serum CRP concentrations at baseline were associated with subsequent incidence of liver cancer and death from chronic liver disease.

Impact: Our findings suggest that levels of systemic inflammation may serve as a long-term marker of liver cancer and liver disease. *Cancer Epidemiol Biomarkers Prev*; 24(2); 386–92. ©2015 AACR.

Introduction

Liver cancer is one of the most common cancers worldwide, and nearly 83% of liver cancers occur in developing countries (1). In China, liver cancer ranks as the second most common cause of cancer deaths (2), with over 383,000 deaths and 395,000 new cases every year (1). Chronic infections with

hepatitis B and C are the predominant causes of liver cancer. Hepatitis B is endemic in China, where approximately 7.2% of the general population are chronically infected, that is, HBsAg positive (3).

Chronic inflammation is thought to be a key mediator of liver cancer, leading to fibrosis and cirrhosis and eventually liver cancer (4, 5). Consistent with this mechanism, recent data from the NIH-AARP cohort suggest that the regular use of aspirin may reduce liver cancer risk (6). C-reactive protein (CRP) is an acute-phase protein that is mainly produced by hepatocytes as part of an inflammatory response. In addition to serving as a marker of systemic inflammation, CRP plays an important role in the inflammatory process where it is involved in opsonization and activation of the complement system in response to IL6 secretion (7). High serum levels of CRP have been associated with the incidence of colorectal and other cancers (8, 9). Among patients with cancer, CRP has also been implicated as a prognostic marker in cancers of the ovary (10), esophagus (11), stomach (12), and colorectum (13).

CRP is produced by the hepatocytes in the liver, and as such could potentially be affected by chronic liver disease. Nevertheless, several recent studies suggest that patients with liver cancer with high CRP levels have poor prognosis (14–17). High CRP levels have also been observed in patients with liver failure (18, 19) and were recently associated with poor prognosis among patients with cirrhosis (20). The long-term associations between CRP and liver cancer and chronic liver disease mortality, however, are not known.

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We prospectively examined the association between serum CRP concentrations and subsequent risk of liver cancer incidence and chronic liver disease mortality in the Chinese Linxian Nutrition Intervention Trials (NIT) cohort.

Materials and Methods

Study population and data collection

Subjects were selected from participants in the Linxian NIT, including the Dysplasia Trial and the General Population Trial. The design and results of the NIT have been described elsewhere (21–23). Briefly, the eligible participants in the Dysplasia Trial were 40- to 69-year old residents with cytologically diagnosed esophageal dysplasia. A total of 3,318 residents from three communes in Linxian were randomized and received either multiple vitamin/mineral supplements (14 vitamins and 12 minerals) or placebos for 6 years, beginning in May 1985.

The eligible participants in the General Population Trial were individuals ages 40 to 69 years from the general population of four communes in Linxian. A total of 29,584 healthy residents were randomized and received up to four daily vitamin/mineral supplement combinations for 5.25 years in a one-half replicate of a 2⁴ fractional factorial experimental design, beginning in March 1986. Individuals who had cancer, debilitating disease including cirrhosis, or required daily medications were excluded from both trials.

At the baseline exams conducted between August 1984 and May 1985, the NIT subjects were interviewed, given a physical examination, and had a 10-mL blood sample drawn. The blood samples were stored on ice for 3 to 6 hours during transport to the field station lab, where the serum was separated, frozen, and stored at –85°C until analyzed. Human subjects protection procedures were approved by the Institutional Review Boards of the U.S. NIH and the Chinese Academy of Medical Science (Beijing, China), and all participants gave written informed consent.

Follow-up for vital status

During the trial and posttrial follow-up periods, we identified incident cancer cases and deaths using several methods that ensured essentially complete ascertainment of events. During the trial period, village health workers visited all subjects monthly, and trial staff reviewed local and regional hospital records and the local cancer registry monthly. A panel of American and Chinese experts confirmed new cancer diagnoses and all causes of death. During the posttrial follow-up period, village doctors continued to visit the participants monthly, and new cancer diagnoses and all causes of death were verified by a panel of Chinese experts. Diagnostic materials evaluated in these expert reviews included pathology and cytology slides, ultrasonography reports, CT reports, clinical histories, biochemical results, and endoscopy and surgery reports. Liver disease deaths, included those caused by cirrhosis and its complications, were determined by the following symptoms: jaundice, ascites, bruising and bleeding, spider angiomas, palmar erythema, gynecomastia and hypogonadism, and combined other materials (biochemical assays and CT scan). Most incident liver cancers were diagnosed by combined evidence from biochemical assays, clinical examination, ultrasound, and CT scan.

Nested case–control design and subject selection

A nested case–control design was used for this study. A total of 255 incident liver cancer cases and 310 nonmalignant chronic liver disease deaths were identified through the end of 2007. Controls were NIT participants who were alive and free of cancer at time of case diagnosis; these controls were frequency matched to the cases 2:1 by age (± 3 years), sex, and trial. CRP was then measured in all of the selected participants who had available serum (incident liver cancer cases: 220, chronic liver disease deaths: 276, controls: 1018). We used the entire set of controls (1,018 subjects) for evaluation of each outcome in the analyses.

Serum CRP measurements

Serum CRP was measured using an electrochemiluminescence assay performed on the Automatic Biochemistry Analyzer (Roche Cobas C501) at the laboratory of the Cancer Institute, Chinese Academy of Medical Sciences. Serum CRP was measured in 42 batches and within each batch, three pooled serum samples were included for quality control (QC) purposes. The mean coefficient of variation of these blinded QC samples for CRP measurements was 2.57% (range: 0.42%–7.85%). For all analyses, the laboratory technicians were blinded to the case–control status of the samples.

Statistical analysis

Baseline demographic characteristics were calculated by case and control groups. Medians and quartiles of serum CRP concentration were calculated by age at baseline (<50, ≥ 50 –<60, ≥ 60), sex, body mass index (BMI), smoking status, drinking status, HBsAg positivity, HBcAg seropositivity, and hepatitis C virus (HCV) seropositivity. The nonparametric Kruskal–Wallis test was used to test the differences of serum CRP concentrations between groups. We used unconditional logistical regression models to estimate ORs and 95% confidence intervals (CI). We also used two different metrics of serum CRP to assess the association between serum CRP concentrations and risk of liver cancer incidence and chronic liver disease mortality: (i) as a continuous variable, scaled to one half the interquartile range $[(0.97-0.17)/2 = 0.40 \text{ mg/L}]$; such that the OR would be per an increase in concentration of 0.40 mg/L and (ii) as quartiles based on sex-specific cutoff values in the controls. Potential confounders included in the fully adjusted models were age (continuous variable), sex (male or female), tobacco smoking (yes (regular cigarette or pipe use for at least 6 months) or no), alcohol drinking [yes (any consumption of alcohol in the previous 12 months) or no], BMI (continuous variable), HBsAg positivity, HBcAg seropositivity, and HCsAg seropositivity. Stratified analyses were conducted by sex, tertiles of follow-up (<7 years, ≥ 7 –<14 years, or ≥ 14 years), and hepatitis B virus (HBV) or HCV infection [HBV positive, defined as HBsAg and/or HBcAg positive (with or without HCV positivity); HCV positive (with or without HBV positivity); or both negative]. When analyses were restricted to women, we excluded the smoking variable from models because of the small number of women who smoked. We also tested *P* values for multiplicative interaction by entering the cross-product of the stratifying exposure and the continuous CRP variable into the regression models and testing whether such an inclusion improved model fit. All statistical analyses were conducted using SAS software (version 9.2, SAS Institute Inc.). All tests were two sided, and *P* < 0.05 was considered statistically significant.

Table 1. Baseline demographic characteristics of case and control groups in the Linxian NIT cohort

	Controls (N = 1,018)	Case (N = 496)			
		Liver cancer incidence (n = 220)	P	Chronic liver disease death (n = 276)	P
Age (y), median (IQR)	55.0 (50.0–61.0)	55.0 (49.0–61.0)	0.72	55.0 (49.0–61.0)	0.83
Sex (%)					
Women	468 (46.0)	88 (40.0)	0.11	137 (49.6)	0.28
Men	550 (54.0)	132 (60.0)		139 (50.4)	
Smoking (%)					
No	657 (64.6)	134 (60.9)	0.30	181 (65.8)	0.71
Yes	360 (35.4)	86 (39.1)		94 (34.2)	
Alcohol drinking					
No	776 (76.3)	165 (75.0)	0.68	213 (77.5)	0.69
Yes	241 (23.7)	55 (25.0)		62 (22.5)	
BMI (kg/m ²), median (IQR)	21.5 (20.2–23.0)	21.8 (20.1–23.0)	0.75	21.6 (20.4–23.3)	0.12
HBsAg (%)					
No	962 (94.6)	168 (76.4)	<0.001	193 (69.9)	<0.001
Yes	55 (5.4)	52 (23.6)		83 (30.1)	
HBcAg (%)					
No	422 (41.5)	67 (30.5)	0.002	82 (29.7)	<0.001
Yes	595 (58.5)	153 (69.5)		194 (70.3)	
HcAg (%)					
No	946 (93.0)	196 (89.1)	0.047	229 (83.0)	<0.001
Yes	71 (7.0)	24 (10.9)		47 (17.0)	
CRP (mg/L), median (IQR)	0.34 (0.15–0.84)	0.47 (0.18–1.16)	0.003	0.54 (0.21–1.36)	<0.001

Results

Two hundred and twenty incident liver cancer cases and 276 chronic liver disease deaths occurred during follow-up, between the baseline exams and December 31, 2007, among NIT participants with available sera. Table 1 presents demographic characteristics of the cases and controls. There were no statistically significant differences between the case and control groups for age at baseline, gender, smoking, drinking, or BMI. As expected, the prevalence of HBsAg, along with HBcAg and HcAg seropositivity was higher in the liver cancer and chronic liver disease groups than in the control group. The median serum CRP level was also significantly higher in the two case groups than in the control group (Table 1).

Serum CRP levels by baseline characteristics in controls are shown in Table 2. Serum CRP levels varied significantly by age at baseline, BMI, and HcAg seropositivity, but were unrelated to gender, male smoking, male drinking, HBsAg, or HBcAg seropositivity.

Table 3 presents the associations between serum CRP concentrations and the risks of liver cancer incidence. There were significant associations between serum CRP levels and risk of liver cancer incidence. Compared with the lowest quartile, subjects in the fourth quartile had a 63% higher risk (OR, 1.63; 95% CI, 1.06–2.51), with evidence of a statistically significant monotonic trend ($P_{\text{trend}} = 0.01$). We also found a significant association between higher serum CRP and higher risk of chronic liver disease mortality (Table 4). Compared with the lowest quartile, subjects in the fourth quartile had a nearly 3-fold higher risk of chronic liver disease deaths (OR, 2.95; 95% CI, 1.90–4.57), with evidence of a statistically significant monotonic trend ($P_{\text{trend}} < 0.001$).

Generally, similar findings were observed in analyses stratified by gender, HBV/HCV status, and trial and we found no statistical evidence for heterogeneity across these subgroups. Similar findings were also found among events occurring close to and many years after CRP measurement. However, we had a modest sample size for these analyses, particularly among HCV-positive participants.

Discussion

This study prospectively examined the association between serum CRP concentrations and risk of liver cancer incidence and death from chronic liver disease. Overall, we observed a significant association between higher serum CRP

Table 2. Serum CRP values (mg/L) in the 25th, 50th, and 75th percentiles of controls in the Linxian NIT cohort, overall, and by baseline characteristics

	25th	50th	75th	P ^a
Overall	0.15	0.34	0.84	
Age at baseline				
<50	0.11	0.20	0.57	<0.001
≥50–<60	0.17	0.35	0.86	
≥60	0.17	0.45	1.07	
Sex				
Women	0.16	0.37	0.90	0.09
Men	0.14	0.32	0.78	
Smoking (male) ^b				
No	0.13	0.29	0.59	0.13
Yes	0.15	0.32	0.86	
Alcohol drinking (male) ^c				
No	0.14	0.28	0.77	0.38
Yes	0.15	0.37	0.78	
BMI (kg/m ²)				
<25	0.14	0.32	0.79	0.002
≥25 to <30	0.26	0.58	1.08	
≥30	0.44	1.22	2.03	
HBsAg				
No	0.15	0.34	0.86	0.16
Yes	0.12	0.26	0.53	
HBcAg				
No	0.14	0.32	0.80	0.82
Yes	0.15	0.35	0.86	
HcAg				
No	0.16	0.35	0.87	0.009
Yes	0.11	0.25	0.49	

^aWe tested for serum CRP concentration differences between groups using the Kruskal–Wallis test.

^bSmoking in males; only one smoker was female.

^cAlcohol drinking in males; only 50 of 241 drinkers were female.

Table 3. Crude and adjusted ORs and 95% CI for associations between serum CRP concentrations and risk of incident liver cancer in a nested case-control study within the NIT cohort

	Continuous ^a	Quartile ^b								<i>P</i> _{trend}	<i>P</i> _{interaction}
		Q1 (n = 247) ^c		Q2 (n = 260) ^c		Q3 (n = 255) ^c		Q4 (n = 256) ^c			
		OR (95% CI)	<i>n</i> ^d	OR (reference)	<i>n</i> ^d	OR (95% CI)	<i>n</i> ^d	OR (95% CI)	<i>n</i> ^d		
Crude	1.00 (0.99-1.02)	43	1.00	47	1.04 (0.66-1.63)	56	1.26 (0.82-1.95)	74	1.66 (1.10-2.51)	0.008	—
Age- and sex-adjusted	1.00 (0.99-1.02)	—	1.00	—	1.04 (0.66-1.62)	—	1.26 (0.81-1.95)	—	1.65 (1.09-2.51)	0.01	—
Fully adjusted ^e	1.00 (0.99-1.02)	—	1.00	—	1.02 (0.64-1.62)	—	1.20 (0.77-1.89)	—	1.63 (1.06-2.51)	0.01	—
Women ^f	0.99 (0.96-1.02)	22	1.00	20	0.88 (0.45-1.72)	20	0.91 (0.46-1.82)	26	1.15 (0.60-2.22)	0.62	0.34
Men ^g	1.01 (0.98-1.04)	21	1.00	27	1.12 (0.58-2.13)	36	1.37 (0.74-2.54)	48	2.00 (1.10-3.62)	0.01	—
Follow-up y ^e											
<7	1.01 (0.98-1.03)	9	1.00	18	1.68 (0.72-3.90)	11	1.01 (0.40-2.56)	23	2.26 (1.00-5.10)	0.11	0.64
≥7-14	1.01 (0.99-1.03)	11	1.00	13	1.05 (0.46-2.42)	16	1.18 (0.53-2.64)	29	2.27 (1.09-4.73)	0.01	—
>14	0.99 (0.95-1.03)	23	1.00	16	0.80 (0.40-1.57)	29	1.50 (0.82-2.76)	22	1.12 (0.59-2.12)	0.36	—
HBV/HCV positivity ^h											
HBV ⁺	1.01 (0.99-1.02)	22	1.00	30	1.36 (0.76-2.43)	45	2.01 (1.16-3.49)	56	2.58 (1.51-4.40)	<0.001	0.37
HCV ⁺	1.01 (0.97-1.05)	5	1.00	1	0.21 (0.02-1.79)	6	1.26 (0.37-4.34)	12	2.84 (0.96-8.41)	0.01	0.81
HBV ⁺ or HCV ⁺	1.01 (0.99-1.02)	23	1.00	30	1.22 (0.69-2.16)	46	1.90 (1.11-3.25)	59	2.49 (1.48-4.19)	<0.001	—
Neither	0.99 (0.96-1.03)	20	1.00	17	0.81 (0.41-1.59)	10	0.51 (0.23-1.12)	15	0.71 (0.35-1.43)	0.21	—
Trial											
Dysplasia trial	1.03 (0.96-1.10)	4	1.00	7	1.89 (0.47-7.63)	9	2.24 (0.56-8.97)	9	2.19 (0.55-8.68)	0.28	0.33
General population trial	1.00 (0.98-1.02)	39	1.00	40	0.95 (0.58-1.55)	47	1.12 (0.69-1.81)	65	1.57 (0.99-2.47)	0.03	—

^aORs for continuous CRP were scaled to one half the interquartile range (0.40 mg/L).

^bWe used sex-specific cutoff values to calculate relative risks. Quartiles for women: <0.16, ≥0.16 to <0.37, ≥0.37 to <0.90, ≥0.90 (mg/L); Quartiles for men: <0.14, ≥0.14 to <0.32, ≥0.32 to <0.78, ≥0.78 (mg/L).

^cNumber of subjects in the control group.

^d*n* cases.

^eAdjusted for age, sex, smoking, drinking, BMI, HBsAg, HBeAg, and HCsAg.

^fAdjusted for age, drinking, BMI, HBsAg, HBeAg, and HCsAg.

^gAdjusted for age, smoking, drinking, BMI, HBsAg, HBeAg, and HCsAg.

^hAdjusted for age, sex, smoking, drinking, BMI, HBsAg/HBeAg, or HCsAg.

concentrations and higher risk of both liver cancer incidence and chronic liver disease mortality. Compared with the lowest quartile, subjects in the fourth quartile of serum CRP had elevated risk of both liver cancer incidence (OR, 1.63; 1.06–2.51) and chronic liver disease deaths (OR, 2.95; 1.90–4.57). The association with incident liver cancer was significant only in men, but the association with chronic liver disease deaths was significant in both genders.

Recent studies suggest that multiple signaling pathways, including NF-κB, c-jun, and STAT3, link chronic inflammation to liver cancer (5) and liver damage-mediated inflammation and carcinogenesis are likely caused by complex cross-talk among these pathways. CRP is an acute-phase reactant, synthesized by hepatocytes in response to systemic inflammation, and is helpful for detecting or predicting outcomes of inflammation. CRP has many pathophysiologic roles in the process of inflammation, including recognizing some foreign pathogens, activating the complement system, initiating the elimination of targeted cells, inducing inflammatory cytokines, and stimulating tissue factor in monocytes (24, 25). CRP may also have an etiologic role in the occurrence of hepatocellular carcinoma. However, the mechanism of CRP action in carcinogenesis in the liver or other organs is largely unclear. Some studies have examined the prognostic value of CRP levels in patients with hepatocellular carcinoma and shown that serum CRP may be a noninvasive prognostic marker for patients with hepatocellular carcinoma (14–17). Although a number of previous studies have investigated associations between serum CRP levels and various cancers (26–31), little epidemiologic data for CRP and liver cancer incidence are available. Only a recent nested case-control study within the European

Prospective Investigation into Cancer and Nutrition cohort found that higher concentrations of CRP were associated with higher risk of hepatocellular carcinoma (incidence rate ratio per doubling of concentrations = 1.22, 95% CI, 1.02–1.46), which was comparable with our findings (32). Little data are also available for the association of CRP with liver disease mortality or incidence, although a recent study observed an association between CRP and short-term outcomes in cirrhosis (20). Additional results suggest elevated levels of CRP in patients with nonalcoholic fatty liver disease (33, 34).

HBV and HCV infections are important risk factors of chronic liver disease and liver cancer. As it is possible that HBV and HCV infection could be associated with CRP levels, we examined associations with liver disease incidence and chronic disease mortality before and after adjustment for HBV and HCV. In our study, however, adjustment for HBV and HCV had little effect on the risk estimates. Such findings suggest that CRP levels may be related to liver cancer incidence in and above effects of these viruses on chronic hepatitis. Nevertheless, future studies are needed to investigate this possibility among populations with a different spectrum of liver disease risk factors, including a low prevalence of HBV and HCV.

One potential concern in this study is the possibility of reverse causality. Because we lacked information about underlying liver disease at baseline and CRP is typically made in the liver, levels could be affected by underlying liver disease. One might expect that individuals with underlying liver disease might therefore have lower levels of CRP. However, higher CRP levels, not lower levels, were associated with subsequent risk of liver cancer and chronic liver disease mortality in the current study, and with

Table 4. Crude and ORs and 95% CIs for the associations between serum CRP concentration and risks of death from chronic liver disease in a nested case-control within the NIT cohort

	Continuous ^a	Quartile ^b								<i>P</i> _{trend}	<i>P</i> _{interaction}
		Q1 (n = 247) ^c		Q2 (n = 260) ^c		Q3 (n = 255) ^c		Q4 (n = 256) ^c			
		OR (95% CI)	<i>n</i> ^d	OR (95% CI)	<i>n</i> ^d	OR (95% CI)	<i>n</i> ^d	OR (95% CI)	<i>n</i> ^d		
Crude	1.01 (1.00-1.02)	37	1.00	56	1.44 (0.92-2.26)	69	1.81 (1.17-2.79)	114	2.97 (1.97-4.48)	<0.001	—
Age- and sex-adjusted	1.01 (1.00-1.02)	—	1.00	—	1.45 (0.92-2.27)	—	1.83 (1.18-2.83)	—	3.03 (2.00-4.58)	<0.001	—
Fully adjusted ^e	1.01 (0.99-1.02)	—	1.00	—	1.44 (0.89-2.31)	—	1.68 (1.06-2.67)	—	2.95 (1.90-4.57)	<0.001	—
Women ^f	1.00 (0.98-1.02)	18	1.00	30	1.67 (0.86-3.26)	37	2.18 (1.13-4.23)	52	3.14 (1.66-5.96)	<0.001	0.39
Men ^g	1.02 (0.99-1.04)	19	1.00	26	1.22 (0.62-2.41)	32	1.29 (0.67-2.49)	62	2.80 (1.52-5.13)	<0.001	—
Follow-up y ^e											
<7	1.02 (1.00-1.04)	12	1.00	9	0.64 (0.26-1.59)	14	0.99 (0.43-2.25)	35	2.52 (1.22-5.19)	0.001	0.54
≥7- $<$ 14	1.01 (0.99-1.03)	15	1.00	22	1.35 (0.67-2.73)	27	1.76 (0.89-3.48)	38	2.52 (1.31-4.82)	0.003	—
>14	1.01 (0.99-1.03)	10	1.00	25	2.50 (1.14-5.45)	28	2.95 (1.36-6.41)	41	5.00 (2.36-10.57)	<0.001	—
HBV/HCV positivity ^h											
HBV ⁺	1.01 (1.00-1.03)	23	1.00	40	1.92 (1.10-3.34)	51	2.41 (1.41-4.14)	83	4.15 (2.48-6.93)	<0.001	0.17
HCV ⁺	1.01 (0.98-1.04)	6	1.00	8	1.28 (0.43-3.83)	17	2.63 (0.99-6.95)	16	2.81 (1.05-7.55)	0.01	1.00
HBV ⁺ or HCV ⁺	1.01 (1.00-1.02)	24	1.00	42	1.69 (0.99-2.87)	54	2.27 (1.35-3.81)	85	3.51 (2.15-5.74)	<0.001	—
Neither	1.01 (0.98-1.03)	13	1.00	14	0.92 (0.42-2.02)	15	0.83 (0.38-1.83)	29	1.76 (0.87-3.54)	0.08	—
Trial											
Dyplasia trial	1.04 (0.97-1.11)	3	1.00	6	1.61 (0.34-7.51)	10	2.12 (0.48-9.34)	10	3.42 (0.79-14.91)	0.08	0.53
General population trial	1.01 (0.99-1.02)	34	1.00	50	1.42 (0.86-2.35)	59	1.63 (0.99-2.66)	104	3.00 (1.89-4.76)	<0.001	—

^aORs for continuous CRP were scaled to one half the interquartile range (0.40 mg/L).

^bWe used sex-specific cutoff values to calculate relative risks. Quartiles for women: <0.16, ≥0.16 to <0.37, ≥0.37 to <0.90, ≥0.90 (mg/L); quartiles for men: <0.14, ≥0.14 to <0.32, ≥0.32 to <0.78, ≥0.78 (nmol/L).

^cNumber of subjects in the control group.

^dNumber of cases.

^eAdjusted for age, sex, smoking, drinking, BMI, HBsAg, HbCag, and HCsAg.

^fAdjusted for age, drinking, BMI, HBsAg, HbCag, and HCsAg.

^gAdjusted for age, smoking, drinking, BMI, HBsAg, HbCag, and HCsAg.

^hAdjusted for age, sex, smoking, drinking, BMI, HBsAg/HbCag, or HCsAg.

nonalcoholic fatty liver disease (33, 34) and poor prognosis among patients with cirrhosis (20) or liver cancer (14-17), as described previously. We examined the associations of CRP levels and liver cancer incidence and chronic liver disease death stratified by tertiles of follow-up years (<7, ≥7- $<$ 14, and ≥14 years). A significant association between higher serum CRP concentrations and liver cancer incidence was seen only in the middle stratum of follow-up (≥7- $<$ 14 years), but a significant association between CRP levels and higher risk of chronic liver disease deaths persisted in cases occurring in all strata, even after 14 years of follow-up. These findings, together with those from previous studies, suggest that elevated CRP levels, likely reflecting systematic inflammation, may serve as a long-term marker of liver cancer and liver disease risk. However, future studies are needed to more comprehensively evaluate inflammatory markers, and further characterize the role of chronic inflammation in both chronic liver disease and liver carcinogenesis.

This study has several strengths, including its prospective design (serum CRP was measured in serum collected before the onset of disease) and its long follow-up time (up to 22 years). We also adjusted for a number of important potential confounders in the multivariate model, including age, sex, smoking, alcohol drinking, BMI, and HBV and HCV positivity. One limitation was that serum levels of CRP, as a marker of systemic inflammation, could also rise due to inflammation in other organs, such as chronic obstructive pulmonary disease (35). However, this potential for reverse causality was minimized because participants who had cancer, debilitating disease, or required daily medications were excluded from the study at baseline. Our sample size was also limited for subgroup analyses, especially among those with HCV positivity. In

addition, we only had a single assessment of baseline CRP levels, which may contribute to misclassification of usual or long-term mean CRP concentration. Finally, only a subset of the incident liver cancer in our study was confirmed by histology. However, we observed the expected associations with HBV and HCV. We would also expect that inclusion of participants without liver cancer in our endpoint would have attenuated the associations that we observed. In addition, as our study population only recently gained access to CT and MRI scanners, it is also possible that the chronic liver disease endpoint included some deaths from undiagnosed liver cancer.

In summary, we found a significant association between higher serum CRP concentrations at baseline and higher risk of both incident liver cancer and chronic liver disease mortality. Furthermore, the association with chronic liver disease mortality persisted even among cases diagnosed years after baseline blood collection. Our results provide further evidence for an important role of inflammation in liver cancer and liver disease progression. However, additional studies in other populations are needed to further evaluate whether levels of systemic inflammation may serve as a long-term marker of liver cancer and liver disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Other (field supervisor): J.-H. Fan

Ethical Approval

This study was approved by the Institutional Review Boards of U.S. NIH and the Chinese Academy of Medical Science, and all NIT participants gave written informed consent for the use of their blood samples and all data.

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