European Prospective Investigation into Cancer and Nutrition, our study provides one of the first pieces of epidemiological evidence on the role of specific biomarkers of inflammation and hyperinsulinemia in the risk of hepatocellular carcinoma (HCC).

The comment by Dr. Li and colleagues implies that by not matching incident cases and controls on hepatitis B and C virus (HBV/HCV) infection, we may have not sufficiently accounted for confounding by HBV/HCV status. We do not agree with this argument. As reviewed in detail elsewhere, the primary purpose of matching in nested case-control studies is not to avoid confounding, but to enhance study efficiency.[2-4] In fact, matching can introduce a selection bias, that must be accounted for in the analysis by control of the matching factors.[4] Our goal was to provide an unbiased estimate of the relative risk of HCC that would be expected in a general population for the metabolic and inflammatory biomarkers. Matching on HBV/HCV would likely make the controls less representative of the person-time experience of the overall cohort[5] and result in biased relative risk estimates. Thus, matching on HBV/HCV infection would not increase the validity (or avoid confounding) of our study. One appropriate method to control for confounding in epidemiological studies is adjustment for potentially confounding variables in regression modeling, as has been done in our study.

In our analysis, adjustment for HBV/HCV infection did not substantially affect the relative risk estimates for the inflammatory and metabolic biomarkers. Thus, the relative risks of HCC per doubling of biomarker concentrations in the final multivariable model for C-reactive protein, interleukin-6, C-peptide, and non-high-molecular-weight adiponectin were 1.10 (95% confidence interval [CI]: 0.95-1.28), 1.79 (95% CI: 1.29-2.46), 2.64 (95% CI: 1.71-4.09), and 2.81 (95% CI: 1.68-4.70), respectively, without adjustment for HBV/HCV infection, and 1.22 (95% CI: 1.02-1.46), 1.90 (95% CI: 1.30-2.77), 2.25 (95% CI: 1.43-3.54), and 2.09 (95% CI: 1.19-3.67), respectively, with adjustment for HBV/HCV infection. Furthermore, as reported in our article, relative risk estimates were not substantially different when persons with prevalent HBV/HCV infection were excluded from the analysis (Supporting Table 2 in our publication). The practical value of our study is supported by our results showing that these inflammatory and metabolic biomarkers significantly improved prediction for future HCC risk beyond HBV/HCV infection and other established HCC risk factors.

In conclusion, we have no doubt in stating that our main inference on the role of inflammatory and metabolic biomarkers in HCC risk is unlikely to be influenced by underlying HBV/HCV infection. We hope that our work will prompt further research that may explore potential applications for cancer prevention.

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Screening for Occult Hepatitis C Virus Infection: Does It Need Special Attention?

To the Editor:

We read with great interest the important article entitled "Unrecognized Chronic Hepatitis C Virus Infection Among Baby Boomers in the Emergency Department" recently published in your esteemed journal.[1] In this cross-sectional study the authors screened 1529 subjects who were born between 1945 and 1965 ("baby boomers") for hepatitis C virus (HCV) infection, and they found 170 persons positive for HCV antibodies. Detection of HCV-RNA and anti-HCV antibodies by enzyme-linked immnunosorbent assay in serum and plasma is the routine diagnostic test for HCV infection.[2] But what about occult HCV infection (OCI)? Here, we want to use this opportunity to make some points about this type of HCV infection.

It is said that in 10% of subjects with abnormal liver function tests the etiology is unknown.[3] A recently defined form of chronic HCV infection, OCI, has been reported as a probable etiology in these cases.[4] This entity is defined by the presence of HCV-RNA in the liver cells without detectable serum HCV-RNA. Also, it can be seen in both positive (with normal liver function tests) and negative (with abnormal liver function tests) anti-HCV antibodies. But in both situations, serum HCV-RNA is usually undetectable.[4-6] Finding HCV-RNA in the liver cells by liver biopsy is the gold standard method for diagnosing OCI. However, instead of this invasive method, it is recommended that detection of HCV-RNA in both peripheral blood mononuclear cells and ultracentrifuged serum is useful. This alternative method helps us to determine about 85% of OCI patients.[5]

Occult HCV infection can lead to a series of complications from minimal changes in liver tissue to liver cirrhosis and finally hepatocellular carcinoma.[7] It has been evaluated and reported in some special groups. Patients on hemodialysis, patients with cryptogenic liver disease, the spouse and other family members of OCI patients, and even healthy subjects without any liver disease are groups where OCI has been reported.[7,8] It is said that in some cases OCI can persist even after treatment, and OCI eradication cannot be achieved in all cases.[9] In the study by Galbraith et al.[1] it is proposed that because of the increased use of illicit drugs and contaminated transfusions that occurred in the 1970s and 1980s, baby boomers are at more risk of HCV infection. Now, with consideration of these

points about OCI, screening of OCI should be taken into account, particularly in special groups like baby boomers.

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Assessing a MicroRNA Panel in Diagnosing Early Cholangiocarcinoma

To the Editor:

Dr. Li and colleagues[1] are to be congratulated on the success in developing a novel method for extracting stable microRNA-laden extracellular vesicles from human bile, determining five microRNA species (miRs) (miR-191, miR-486-3p, miR-1274b, mi-R 16, and miR-484) using computer-based classifiers, and discriminating cholangiocarcinoma (CCA) from benign biliary diseases with a five-miRNA panel. A sensitivity of 67% and a specificity of 96% would be sufficient to use the panel in clinical practice, although use of the panel may be time-consuming and expensive. Li et al. conclude that their panel is superior to CA 19-9 in detecting early CCA, because the panel diagnosed more N0M0 tumors than CA 19-9 and two patients were correctly diagnosed as having a T1N0M0 tumor only by the panel. The outcome of surgery for CCA is affected by not only nodal status but also tumor size and tumor stage. The patients suffering from stage 1 or stage 2 intrahepatic cholangiocarcinoma (ICC) had a significantly better postoperative outcome than stage 3 patients and all other patients.[2] When looking at table 1B,[1] a sensitivity of the panel (55%) is the same as that of CA 19-9 in stage 1 (T1N0M0) and stage 2 (T2N0M0) tumors. Further studies enrolling stage 1/2 CCAs would be warranted because the diagnostic accuracy of an enhanced computed tomography (CT) scan and magnetic resonance imaging (MRI) for larger liver tumors is high.[3]

Among the five miRs making up the panel in the study by Li et al., miR-191 was found to be highly expressed also in hepatocellular carcinomas (HCCs) taken from patients, and was shown to be a potential therapeutic target.[4] Conversely, higher expression of miR-486-3p in nontumor liver tissues in patients with HCC was associated with a better survival, meaning that miR-486-3p might down-regulate the growth of HCC.[5] Despite advances in noninvasive diagnosis of liver nodules, it is difficult to discriminate a small (<2 cm) ICC from other small liver nodules such as HCC, focal fatty infiltration, arterioportal shunt, immature abscesses, macroregenerative nodules, hepatic tuberculosis, focal nodular hyperplasia, dysplastic nodules, hemangiomas, and metastatic nodules from other sites. Since some miRs are likely involved in the regulation of both ICC and HCC, it would be interesting to investigate whether the panel is applicable to discrimination between small ICCs and small HCCs.

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