

Does occult HBV infection have an impact on the evolution of chronic hepatitis C?

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Viral co-infections with HBV or HIV are well known to aggravate the evolution of chronic hepatitis C (CHC). With respect to HBV, although the impact of overt infection has been well characterized, the role of occult HBV infection (OBI) on the outcome of CHC remains elusive [1,2]. OBI are mainly defined by the persistence of HBV genome in the liver of individuals testing negative for HBsAg [1]. The mechanisms by which the persistence of the viral genome is not associated with a full expression of viral antigens and viral replication are still partially known [3]. The pathobiological and clinical consequences of HBV genome persistence as an OBI, whether as a mono-infection or as a co-infection with HCV, include an increased severity of liver disease and an increased risk of HCC [4]. However, most of the published studies were cross-sectional and were therefore subjected to potential bias in patient selection.

OBI is highly prevalent in patients with CHC, at least in areas of high HBV prevalence. Whether OBI might negatively influence CHC clinical outcome, favoring its progression toward cirrhosis, has been largely debated for many years. Controversies remain on the possible prooncogenic role exerted by OBI, particularly in CHC patients [5]. A recent meta-analysis suggested that OBI increases the risk of hepatocellular carcinoma (HCC) development in both HCV and non-HCV infected patients [6], but other studies performed in North America did not find such an association [7].

One main reason for the discussion of the possible contribution of OBI to a more severe evolution of CHC is the lack of longitudinal studies evaluating the clinical outcome of HCV patients according to the status of OBI during a sufficient period of observation to allow clinical events to occur.

In this paper, Squadrito *et al.* [8] have performed a long awaited observational cohort study evaluating the clinical evolution of CHC patients according to their OBI status. From 1991 to 2000, 326 HBsAg negative CHC patients were tested for OBI by the analysis of HBV genome by PCR in liver biopsy samples. A total of 128/326 cases (39.2%) tested OBI positive and 198/326 (60.8%) OBI negative. Ninety-four of 326 patients (37 OBI positive, 57 OBI negative) were followed-up for a median time of

11 years, which was an important asset of the study. During the follow-up, 79/94 patients underwent anti-HCV treatments and 26 achieved a sustained virological response that occurred independently of their OBI status. Eighteen patients (13/37 OBI positive, 5/57 OBI negative, $p < 0.01$) developed HCC. Among the 76 non-HCC individuals, 15 subjects (8/24 OBI positive, 7/52 OBI negative, $p < 0.05$) developed advanced forms of cirrhosis. Eighteen patients died during the follow-up and 2 underwent liver transplantation. OBI positive individuals had a cumulative survival rate significantly shorter than OBI negative individuals ($p = 0.003$). Liver related deaths were more frequently found in OBI positive than OBI negative patients (12/37 OBI positive vs. 6/57 OBI negative patients, respectively, $p < 0.01$). Finally, non-response to anti-HCV therapy was significantly associated with lower survival ($p = 0.02$). Therefore, the authors concluded that among CHC patients, OBI co-infected individuals are at high risk of progression toward cirrhosis, HCC development, and lower survival.

Although this study was not designed as a true prospective study, it is an important evaluation over time of a cohort of unselected HCV patients who underwent a liver biopsy and tested for OBI in a single liver unit in Sicily in the 90s. This is the first longitudinal cohort addressing this question. The authors tried to carefully rule out an influence of alcohol consumption and cigarette smoking as potential cofactors influencing the outcome of CHC [9]. They also considered the potential role of liver steatosis on the analysis of liver histology at baseline to assess the possible dependence of the events occurring in this HCV cohort, but did not show any correlation with a severe outcome of the disease. It is noteworthy that in OBI patients who developed HCC or severe worsening of liver disease, only a minority had cirrhosis at the time of biopsy (4 among HCC patients, 2 being OBI positive, and 6 among patients with progressive liver disease, 3 being OBI positive), suggesting an important role of occult HBV in the progression of liver disease and occurrence of clinical events.

Unfortunately, data evaluating the rate of progression of the liver disease are lacking since a second liver biopsy was performed only in a small number of cases during follow-up, when it was clinically relevant, while non-invasive approaches assessing fibrosis progression, such as fibroscan, were not available for the majority of patients. In that respect, it would be interesting to study, in a retrospective manner, blood markers of liver fibrosis on stored serum samples.

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This important clinical study provides new information to our knowledge of the potentiating role of OBI in the evolution of liver disease in the context of CHC. This opens new questions regarding the pathobiology of these co-infections. Up to now, there was no suitable cell culture system to analyze a true co-infection with both HCV and HBV. Primary hepatocytes or HepaRG cells which are susceptible to HBV infection do not yet support robust levels of HCV replication. A HuH7.5 cell line stably transfected with the HBV genome was transfected with HCV replicons or infected with HCVcc, but results did not show direct viral interference [10]. With the discovery of sodium taurocholate cotransporting polypeptide (NTCP) as a receptor for HBV infection, it will now be possible to study the pathogenesis of true co-infections in this transformed hepatoma cell line [11,12]. New technological developments will be necessary to obtain non-transformed hepatocyte cell culture systems susceptible to both viruses to study the impact of their interactions on hepatocyte biology at the molecular level; in this respect, the recently published model based on human induced pluripotent stem cells (iPSCs) offers the ability to produce host-specific differentiated cells that might be susceptible to infection by both viruses [13]. On a clinical point of view, it would be important to obtain independent confirmation of these results in other cohorts of patients from different regions of the world, where HBV infection is highly prevalent. Indeed, if confirmed, this would have major clinical implications in terms of diagnosis of OBI, which would then require the development of standardized assays. Another important impact would be on the management of patients who would need more aggressive treatment intervention. However, there is currently no proven beneficial effect of anti-HBV therapy on OBI itself. Interestingly, the finding that patients who cleared HCV infection upon anti-HCV therapy had a better clinical outcome is reassuring.

In conclusion, this is an important longitudinal clinical study showing the role of occult HBV infection in the progression of chronic hepatitis C in an Italian cohort of patients. Further studies are warranted to better understand the molecular basis of liver pathogenesis in the setting of this viral co-infection and to better define the clinical management of these patients.

Conflict of interest

The author declared that he does not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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