CURRENT TREND

Hepatitis B: Liver fibrosis and hepatocellular carcinoma

Hépatite B : fibrose hépatique et carcinome hépatocellulaire

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Summary  Chronic hepatitis B virus (HBV) infection is estimated to be the cause of 55–60% of hepatocellular carcinoma (HCC) in the world. It has been estimated that up to 40% of HBV-related HCC occur in persons who do not have cirrhosis while almost all cases of hepatitis C virus (HCV)-related HCC occur in the setting of cirrhosis. Data on the performance of non-invasive tests for liver fibrosis in patients with hepatitis B are limited. FibroTest may be superior to the Forns index, APRI, Goteborg University Cirrhosis Index (GUCI) and Hui model in detecting significant fibrosis (Metavir > F2) or cirrhosis (Metavir F4) but an algorithm that uses APRI for screening, FibroTest for confirmation, and biopsy for indeterminate cases has the greatest accuracy. Liver stiffness correlates with fibrosis stages but may be influenced by necroinflammatory activity with falsely high values in patients with alanine aminotransferase (ALT) flares and falsely low values in patients with viral suppression and ALT normalization during antiviral therapy. Therefore, additional studies are needed to determine the clinical settings in which liver stiffness measurement can accurately predict liver fibrosis and to establish cutoff values for differentiating different stages of fibrosis or cirrhosis. These studies should also compare the performance of liver stiffness measurement with serum markers of fibrosis in patients with varying degrees of necroinflammation and in untreated patients as well as patients receiving antiviral therapy. Until recently, older age, male gender and cirrhosis were the major risk factors associated with HCC development. Recent studies showed that HBV replication status, HBV genotype and mutations in the basal core promoter region play an important role in HCC development. These data indicate that algorithms incorporating demographics, viral factors, degree of necroinflammation and extent of fibrosis may be more accurate in predicting the risk of HBV-related HCC than fibrosis staging alone.

Résumé  Dans le monde, l’infection chronique par le virus de l’hépatite B (VHB) serait la cause de 55 à 60 % des cas de carcinome hépatocellulaire (CHC). Près de 40 % des CHC liés à
Introduction

Chronic hepatitis B virus (HBV) infection is estimated to be the cause of 55–60% of hepatocellular carcinoma (HCC) in the world. The annual incidence of HCC has been estimated to be less than 1% for non-cirrhotic carriers and 2%–3% for patients with cirrhosis [1]. Several lines of evidence indicate that the incidence of HCC is higher among Asians than Caucasians. This difference may be partly related to a longer duration of infection among Asians (perinatal vs. adult acquired infection) but several studies suggest that other factors such as HBV genotype and exposure to aflatoxin may contribute to this difference. This article will review:

- the frequency in which HBV-related HCC is found in the absence of cirrhosis;
- the utility of non-invasive tests for assessment of hepatic fibrosis or cirrhosis;
- the risk factors for HCC in persons with chronic HBV infection.

HBV-related HCC in patients without significant fibrosis or cirrhosis

Cirrhosis, regardless of etiology, is considered to be the most important risk factor for HCC. It has been estimated that up to 40% of HBV-related HCC occur in persons who do not have cirrhosis while almost all cases of hepatitis C virus (HCV)-related HCC occur in the setting of cirrhosis. The explanation for the more frequent occurrence of HBV-related HCC in the absence of cirrhosis has been attributed to the direct oncogenic effects of HBV. It should be emphasized that the proportion of HCC occurring in a non-cirrhotic liver is difficult to ascertain because liver histology is not available in all HCC cases and among those who underwent liver biopsy, the adjacent non-tumorous liver tissue may not be adequate for fibrosis assessment. Therefore, data on the frequency in which HCC occurs in a non-cirrhotic liver have relied on studies of patients who underwent surgical resection.

Retrospective analysis of patients who underwent surgical resection

In a retrospective analysis of 330 HCCs resected in a French hospital between 1985 and 1998, 250 (76%) showed cirrhosis (n=158) or extensive fibrosis (n=92) while 52 (16%) had minimal portal fibrosis and 28 (8%) had no fibrosis in the non-tumorous liver [2]. HCC was found in the setting of no or minimal fibrosis in 18% (17/96) of patients with chronic HBV infection and in 27% (63/234) of patients with other causes of liver disease. Another study from Hong Kong found that 44% of patients under or equal to 40 years and 47% of those over 40 years who underwent surgical resection for HBV-related HCC did not have cirrhosis in the non-tumorous liver [3]. These data indicate that HCC can occur in a non-cirrhotic liver but the frequency in which this occurs is unclear and may be heavily biased by the criteria used for selecting patients for surgical resection. It should be noted that HCCs in non-cirrhotic livers have also been observed in patients with other causes of liver disease including hepatitis C and presumed nonalcoholic fatty liver disease [4,5].

Prospective cohort studies

Cohort studies have also been used to estimate the risk of HCC in hepatitis B surface antigen (HBsAg) positive persons...
with and without cirrhosis. In one study, 22,707 Taiwanese men including 3454 who were HBsAg positive were followed for a mean of 8.9 years. Among the HBsAg-positive persons, 40 were known to have cirrhosis at enrollment and their incidence of HCC was 4.7-fold higher than those with no known cirrhosis [6]. In another study, the REVEAL-HBV study, 3653 HBsAg-positive and hepatitis C antibody negative persons recruited from various communities in Taiwan were followed for a mean of 11.4 years [7]. The incidence of HCC per 100,000 person years was 6482 among the 69 persons with cirrhosis and 317 among those with no cirrhosis with an adjusted hazard ratio for HCC among those with cirrhosis of 9.1 (95% CI 5.9—13.9) (p < 0.001). It should be noted that only 69 persons had cirrhosis in this study and the diagnosis of cirrhosis was based on ultrasound findings at one time point only (time of enrollment).

Non-invasive tests for hepatic fibrosis or cirrhosis

Liver biopsy is the gold standard for assessment of hepatic fibrosis or cirrhosis but it is an invasive procedure with a risk of significant bleeding of one in 2500 to one in 10,000 and a risk of death of less than or equal to one in 10,000 and is subject to sampling error [8]. During the last 15 years, there has been extensive research into non-invasive tests for hepatic fibrosis or cirrhosis. These tests include indices or algorithms based on routine laboratory tests, panels of serum fibrosis markers, liver stiffness measurement, and radiologic imaging [9,10]. Most of the studies were performed in patients with hepatitis C. These studies showed that non-invasive tests are more accurate in detecting cirrhosis than in differentiating different stages of fibrosis and liver stiffness is more accurate than blood tests. Data on the performance of these non-invasive tests in patients with hepatitis B are limited.

Biomarkers or serum indices of hepatic fibrosis

In one study, FibroTest and ActiTest were evaluated in 462 of 695 hepatitis B patients randomized in two pivotal studies of adefovir therapy who had adequate paired biopsies and stored sera within 180 days of the biopsy [11]. The FibroTest comprised five markers: alpha2-macroglobulin, haptoglobin, gamma glutamyl transpeptidase, total bilirubin and apolipoprotein A1 while the ActiTest comprised these same markers plus alanine aminotransferase (ALT) [12]. The area under the receiver operating characteristic curve (AUROC) of FibroTest for diagnosing advanced fibrosis (Metavir F2/F3/F4) was 0.76 (95% CI 0.73—0.80) and for the diagnosis of cirrhosis (Metavir F4) was 0.82 (95% CI 0.77—0.86). The AUROC for FibroTest for diagnosing advanced fibrosis were similar in patients with normal (0.77, 95% CI 0.71—0.78) or abnormal ALT (0.76, 95% CI 0.72—0.79) (p = 0.75). The ActiTest had an AUROC of 0.81 (95% CI 0.78—0.83) for the diagnosis of moderate—severe necroinflammatory activity (Metavir A2/A3) and was higher than the AUROC for ALT (0.71, 95% CI 0.68—0.74) (p < 0.0001). The authors also showed that histologic response (decrease in Knodell necroinflammation and no worsening of fibrosis) to adefovir correlated with an improvement in ActiTest. Furthermore, they found that the FibroTest and the ActiTest performed equally well in predicting fibrosis and necroinflammation in liver biopsies obtained before and after one year of treatment.

Another study of 137 patients in China found that the aspartate aminotransferase platelet ratio index (APRI) [13] correlated with fibrosis stage. An APRI greater than or equal to 1.5 had a sensitivity of 44.7% and a specificity of 84.3% in detecting advanced fibrosis (stages 2—4), and addition of hyaluronic acid increased the specificity to 98.9% when a cutoff of 300 ng/mL was used [14].

A third study of 110 patients in Italy found that FibroTest was superior to the Forns index [15], APRI, Goteborg University Cirrhosis Index (GUCI) [16] and Hui model [17] in detecting significant fibrosis (Metavir ≥ F2) or cirrhosis (Metavir F4) but an algorithm that uses APRI for screening, FibroTest for confirmation, and biopsy for indeterminate cases had the greatest accuracy [18].

Measurement of liver stiffness

Several studies have also evaluated the utility of liver stiffness measurement by transient elastography in assessing liver fibrosis. In one study, 161 patients were included; of which 37 had Metavir F3 and 40 had F4. The median liver stiffness measurements for patients with F0, F1, F2, F3, and F4 were 5.9, 5.9, 7.0, 8.8, and 14.2 kPa [19]. The AUROC for differentiating F0 vs. F1—F4 was 0.80 (95% CI 0.68—0.92, p = 0.002), F0—2 vs. F3—4 was 0.87 (95% CI 0.82—0.93, p < 0.001), and F0—3 vs. F4 was 0.93 (95% CI 0.89—0.97, p < 0.001). Based on these findings, the authors proposed that a liver stiffness measurement greater than or equal to 8.4 kPa which had a sensitivity of 90% be considered as possible cirrhosis and a measurement greater than or equal to 13.4 kPa which had a specificity of 94% as probable cirrhosis. The authors also noted that patients with elevated ALT had higher liver stiffness measurements than those with normal ALT at the same stage of liver fibrosis. The median liver stiffness measurements for patients with elevated ALT vs. normal ALT were 6.3 vs. 4.7 kPa (p = 0.029) for patients with F0 and 16.6 vs. 12.3 kPa for patients with F4 (p = 0.02). In another study by the same investigators, 1197 hepatitis B e antigen (HBeAg)-negative patients underwent liver stiffness measurement. The authors reported that the risk of cirrhosis increased with serum HBV DNA and ALT values [20]. However, it is unclear if the findings reflect a genuine increase in risk of cirrhosis or a falsely high liver stiffness measurement in patients with high ALT and / or high serum HBV DNA. In a third study, 188 patients not receiving antiviral therapy and 80 patients receiving antiviral therapy were evaluated [21]. The authors found that for each stage of fibrosis, patients who were receiving antiviral therapy had lower liver stiffness measurements than those who were not on treatment; the mean values for Ishak fibrosis stage 0—2 were 6.1 vs. 6.4 kPa, Ishak 3—4 were 8.5 vs. 10.1 kPa, and Ishak 5—6 were 11.7 vs. 15.7 kPa, respectively. In this same study, liver stiffness measurement increased 1.2 to 4.4-fold during hepatitis flares, and serial measurements showed that changes in liver stiffness paralleled changes in ALT.

Available results indicate that liver stiffness correlates with fibrosis stages in patients with chronic HBV infection but liver stiffness values may be influenced by necroinflam-
matory activity with falsely high values in patients with ALT flares and falsely low values in patients with viral suppression and ALT normalization during antiviral therapy. Given the fluctuating nature of chronic HBV infection, the confounding effect of ALT flares and hepatic necroinflammation on liver stiffness measurement is likely more pronounced in patients with hepatitis B than those with hepatitis C. Therefore, additional studies are needed to determine the clinical settings in which liver stiffness measurement can accurately predict liver fibrosis and to establish cutoff values for differentiating different stages of fibrosis or cirrhosis. These studies should also compare the performance of liver stiffness measurement with serum markers of fibrosis in patients with varying degrees of necroinflammation and in untreated patients as well as patients receiving antiviral therapy.

**Risk factors for HBV-related HCC**

Until recently, older age, male gender and cirrhosis were the major risk factors associated with HCC development. Recent studies showed that HBV replication status, HBV genotype and mutations in the basal core promoter region play an important role in HCC development. These data indicate that algorithms incorporating demographics, viral factors, degree of necroinflammation and extent of fibrosis may be more accurate in predicting the risk of HBV-related HCC than fibrosis staging alone [22].

**HBeAg status and serum HBV DNA level**

Several studies have reported an association between persistent HBeAg seropositivity and/or reversion to HBeAg positivity after initial HBeAg seroconversion and HCC development. A community-based prospective study of 2361 HBsAg-positive men aged 30—65 years followed for a mean of 8.5 years found that the adjusted relative risk of HCC for persons who were HBeAg-positive at entry was six to seven-fold higher than those who were HBeAg-negative [23]. A nested case-control analysis of those who were HBeAg-negative showed that the risk of HCC was related to serum HBV DNA levels with odds ratio for HCC development of 2.3 for those with baseline HBV DNA 2.5—13 pg/mL and 6.0 for those with HBV DNA greater than 13 pg/mL.

A dose-response relationship between serum HBV DNA levels and the risk of HCC development has been confirmed by other studies. In the REVEAL study, 2260 HBsAg-positive men and 1393 HBsAg-positive women aged 30—65 years in Taiwan were followed for a mean of 11.4 years. Baseline serum HBV DNA (> 10^4 copies/mL) was a strong predictor of HCC independent of HBeAg status, ALT level, and presence of cirrhosis [7]. Another study of HBsAg-positive Taiwanese men included 154 HCC cases and 316 matched controls, aged 30 years or older that the odds ratio for HCC among those with serum HBV DNA level in the highest quintile was 7.26 compared to those with serum HBV DNA level in the lowest quintile [24]. A third study of 2763 HBsAg-negative persons in China found that compared to those with serum HBV DNA less than 1600 copies/mL, persons with HBV DNA up to 5 log_{10} copies/mL and those with HBV DNA greater than 5 log_{10} copies/mL had adjusted risk of HCC-related deaths of 1.7 (95% CI 0.5—5.7) and 11.2 (95% CI 3.6—35.0), respectively [25]. Studies in Senegal and Japan also confirmed an increased risk of HCC among carriers with high baseline serum HBV DNA levels.

To date, most studies focused on baseline HBV DNA levels. Given the fluctuating course of chronic HBV infection, the duration of high levels of HBV replication as well as the intensity and frequency of hepatitis activity may be more important than a single high HBV DNA level in predicting the risk of HCC. Indeed, in the REVEAL study, when paired HBV DNA levels at entry and at last follow-up were considered, the risk of HCC was significantly increased only in individuals with serum HBV DNA exceeding 5 log_{10} copies/mL in one sample and 4 log_{10} copies/mL in the other sample but not in those with serum HBV DNA between 4—5 log_{10} copies/mL in both samples [7]. Preliminary analysis using a time dependent Cox regression analysis based on serial serum HBV DNA levels found that the adjusted hazard ratio for HCC was significantly higher in patients with HBV DNA greater than or equal to 6 log_{10} copies/mL [26].

**HBV genotypes and core promoter variants**

Studies in Asia found that HBV genotype C is more prevalent in patients with HCC than those with genotype B infection. The association between genotype C and an increased risk of HCC has also been confirmed in several longitudinal studies. In one study in Taiwan, a total of 153 HCC cases occurred during 33,847 person-years of follow-up. The HCC incidence rates per 100,000 person-years for participants infected with HBV genotype B or C were 306 (95% CI 237—388) and 786 (95% CI 627—973), respectively [27]. The association between genotype C and HCC may be related to delayed HBeAg seroconversion and a longer period of active HBV replication [28]. In addition, genotype C is more frequently associated with core promoter mutations [29] which have been shown to be an independent predictor of HCC. A study from Taiwan found that the adjusted hazard ratio for HCC after adjusting for other risk factors, including HBV genotype was 1.73 (95% CI 1.13—2.67) among HBsAg-positive persons with core promoter mutations (A1762T/G1764A) compared to those with wild-type sequence in the core promoter region [27].

Studies outside Asia have also observed an association between HBV genotypes and HCC. A study in sub-Saharan Africa showed that genotype A was associated with an increased risk of HCC [30] while a study in Alaska natives reported that genotype F was associated with the highest incidence of HCC [31].

**Conclusion**

Most cases of HBV-related HCC occur in the setting of cirrhosis but HBV-related HCC can occur in non-cirrhotic livers. Besides cirrhosis, host and viral factors contribute to the risk of HCC. Data on non-invasive assessment of liver fibrosis in persons with chronic HBV infection are limited. Available data suggest that biomarkers and serum panels of routine laboratory tests / fibrosis makers have similar accuracies in predicting advanced fibrosis or cirrhosis in persons with HBV infection as those with HCV infection. However, liver stiffness measurement may be affected by inflammation...
and its accuracy in predicting fibrosis or cirrhosis in persons with HBV infection may be lower than in those with HCV infection. Contrary to hepatitis C where cirrhosis is the most important risk factor for HCC, various viral factors including HBV genotype, core promoter mutations and HBV replication status play a key role in HCC development.

Conflicts of interest

None.

References

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