Negative HbcAg in immunohistochemistry assay of liver biopsy is a predictive factor for the treatment of patients with nucleos(t)ide analogue therapy

Mingxing Huang a, Jian Liu a, Monica Chow b, Xuan Zhou a, Zongping Han a, Zhenjian He c, Jinfang Xue a, Zhe Zhd, e, Xinhua Lif, *, Jinyu Xia a, *

a Department of Infectious Diseases, The Fifth Affiliated Hospital of Sun Yat-Sen University (SYSU), Zhuhai, Guangdong, China
b PGY IV, Department of General Surgery Rutgers, Robert Wood Johnson Medical School, Piscataway, NJ, USA
c School of Public Health, Sun Yat-sen University, Guangzhou, China
d Department of Medicine, Division of Regenerative Medicine, University of California, San Diego, School of Medicine, La Jolla, CA, USA
e Department of Stem Cell Biology and Regenerative Medicine, Cleveland Clinic, Lerner Research Institute, Cleveland, OH, USA
f Department of Infectious Diseases, The Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou, Guangdong, China

Received: July 19, 2017; Accepted: September 18, 2017

Abstract

The hepatitis B core antigen (HbcAg) is an important target for antiviral response in chronic hepatitis B (CHB) patients. However, the correlation between HbcAg in the hepatocyte nucleus and nucleos(t)ide analogue (NA) therapeutic response is unclear. We sought to evaluate the role of HbcAg by analysing liver biopsies for viral response in NA-naive hepatitis B e antigen (HBeAg) positive (+) CHB patients via immunohistochemistry (IHC). A total of 48 HbcAg-negative (−) patients and 48 HbcAg (+) patients with matching baseline characteristics were retrospectively analysed for up to 288 weeks. Virological response (VR) rates of patients in the HbcAg (−) group were significantly higher at week 48 and 96 than the HbcAg (+) group (77.1% versus 45.8% at week 48, respectively, P = 0.002 and 95.3% versus 83.3% at week 96, respectively, P = 0.045). The serological negative conversion rate of HBeAg was significantly higher in the HbcAg (−) than in the HbcAg (+) group from week 96 to 288 (35.4% versus 14.6% at week 96, respectively, P = 0.018; 60.4% versus 14.6%, respectively, P < 0.001 at week 144; 72.9% versus 35.4%, respectively, P < 0.001 at week 288). The cumulative frequencies of VR and lack of HBeAg were higher in the HbcAg (−) group (both P < 0.05). Binary logistic regression analysis showed that HbcAg (−) was the predictor for the lack of HBeAg (OR 4.482, 95% CI: 1.58–12.68). In summary, the absence of HbcAg in the hepatocyte nucleus could be an independent predictor for HBeAg seroconversion rates during NA-naive treatment in HBeAg (+) CHB patients.

Keywords: hepatitis B core antigen ● hepatitis B e antigen ● immunohistochemistry ● chronic hepatitis B ● nucleos(t)ide analogue

Introduction

Hepatitis B virus (HBV) is still a major global health problem and the leading cause of liver diseases, cirrhosis and hepatocellular carcinoma [1], with an estimate of 240 million patients worldwide [2]. As a result, more than 686,000 people die every year due to complications caused by hepatitis B [1–4].

Active replication of HBV is indicated by the expression of hepatitis B e antigen (HBeAg) [5], which is closely associated with an increased risk of hepatocellular carcinoma [6]. Loss of HBeAg expression and/or neutralization of HBeAg by antibodies has been shown to be an end-point for treatment related to chronic hepatitis
were recorded for each patient prior to treatment. Levels and time to HBeAg (status/levels, time to ALT normalization, time to undetectable HBV DNA quantities of hepatitis B surface antigen (HBsAg), HBeAg and anti-HBe activity: 18% (125/138) and 21.7% (30/138) seroconversion rate at weeks 48 and 96, respectively [11].

Hepatitis B core antigen (HBcAg) is also known to be an important marker for the proliferation of HBV [12]. HBcAg is coded by gene C of HBV, which is essential to viral replication [13]. From immunohistochemical (IHC) staining patterns, HBcAg can be classified as having cytoplasmic expression (cHBcAg), nuclear expression (nHBcAg), cytoplasmic and nuclear-mixed expression (mHBcAg) or negative expression [14]. The different distributions of HBcAg expression in the hepatocyte nucleus and cytoplasm of CHB indicate the level of viral replication and histological activity [12–14]. HBcAg is localized mainly in the nucleus in the viral replicative stage and in the cytoplasm of hepatocytes during the viral clearance phase [15, 16]. Serum Hbc antigen concentration is positively correlated with intrahepatic covalently closed circular DNA (cccDNA) levels [17]. Therefore, HBcAg in the hepatocyte nucleus also may positively correlate with intrahepatic cccDNA levels, which could serve as another important predictor for virological response during antiviral therapy for CHB patients [18].

There have been limited reports detailing the role of hepatocyte nuclear HBcAg in antiviral therapy. The aim of this study was to investigate the role of hepatocyte nuclear HBcAg in HBeAg-positive (+) NA-naive CHB patients during NA therapy. We examined the viral response rates and the serum HBeAg-negative (−) rates of HBcAg (−) and HBcAg (−) patients using IHC on liver biopsies.

Materials and methods

Patient selection

All the selected patients in our study group were from the 5th Affiliated Hospital, Sun Yat-sen University (SYSU). The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the 5th Affiliated Hospital Ethical Committee at SYSU. The study design and manuscript preparation fully followed the guidelines from the STROBE statement [19]. Written informed consent was obtained from all patients.

From 1 January 2010 to 31 July 2016, 96 NA-naive CHB patients with serum positive for HBeAg were enrolled. For patients with HBV infection, parameters including age, sex, serum alanine aminotransferase (ALT), HBV DNA levels at baseline and throughout treatment, quantities of hepatitis B surface antigen (HBsAg), HBeAg and anti-HBe status/levels, time to ALT normalization, time to undetectable HBV DNA levels and time to HBeAg (+) to (−) seroconversion during follow-up were recorded for each patient prior to treatment. Patients were excluded from this study if they (1) were serum HBeAg negative, (2) were coinfected with other hepatitis viruses or had other comorbidities, (3) displayed alcohol-induced or autoimmune liver diseases, (4) were pregnant or lactating and (5) had been treated with NA previously.

Patients were divided into two groups, HBeAg (−) and HBeAg (+), based on the lack of or presence of HBeAg in hepatocyte nucleus, respectively. A total of 48 HBeAg (−) patients and 48 HBeAg (+) patients matched in sex, age and baseline HBV DNA levels were included in this study (Table 1; Fig. 1). Histological inflammatory activity (determined by simple grading and staging systems for chronic viral or autoimmune hepatitis) [20] and IHC of liver biopsies were measured before starting NA therapy. Patients followed up every 3 to 6 months for blood collection and liver ultrasound examination for a total of 288 weeks. All the patients were treated according to the guidelines of prevention and treatment for CHB (2010 edition) [21].

| Items | HBeAg (−) | HBeAg (+) | P |
|-------|---------|---------|---|
| Age, year | 37 (22–59) | 35 (23–59) | 0.712 |
| Gender, M% | 85.4 (41/48) | 79.2 (38/48) | 0.423 |
| ALT, U/l | 115.6 ± 157.6 | 170.2 ± 320.8 | 0.286 |
| HBV DNA, log10 IU/ml | 6.02 ± 1.27 | 6.28 ± 1.18 | 0.311 |
| HbsAg, Log10 IU/ml | 3.14 ± 0.61 | 3.09 ± 0.65 | 0.660 |
| HBeAg, Log10 S/CO | 1.82 ± 0.52 | 1.90 ± 0.75 | 0.476 |
| HbcAb−, Log10 S/CO | 1.23 ± 0.14 | 1.18 ± 0.18 | 0.179 |

Knodell necroinflammatory score*

| Grade | HBeAg (−) | HBeAg (+) | P |
|-------|---------|---------|---|
| Grade 1 | 4 (8.3%) | 8 (16.7%) | |
| Grade 2 | 20 (40.4%) | 31 (64.6%) | |
| Grade 3 | 15 (31.3%) | 9 (18.7%) | 0.280 |

Fibrosis*

| Stage | HBeAg (−) | HBeAg (+) | P |
|-------|---------|---------|---|
| Stage 0 | 8 (16.7%) | 10 (20.8%) | |
| Stage 1 | 8 (16.7%) | 7 (14.6%) | |
| Stage 2 | 8 (16.7%) | 9 (18.7%) | |
| Stage 3 | 7 (14.5%) | 9 (18.7%) | |
| Stage 4 | 17 (35.4%) | 13 (27.1%) | 0.900 |

HbcAb reference: 1.0000–3.0000 negative and 0–1.0000 positive. HBeAg reference: <1 negative and >1 positive. HbcAb reference: <1 positive and >1 negative. ALT: Alanine transaminase.

*Simple grading and staging systems for chronic viral hepatitis [24].
NA therapy and detection methods

All the patients received daily NA therapy (including lamivudine (LAM), adefovir dipivoxil (ADV), telbivudine (LDT), entecavir (ETV) or ADV combined with either LAM or ETV). A liver biopsy was taken from each patient prior to treatment initiation. HBsAg and HBCAg in the hepatocyte nucleus were measured using IHC staining with mouse anti-human HBsAg monoclonal antibody and rabbit anti-human HBCAg polyclonal antibody, respectively. Staining was performed according to the manufacturer’s protocols (Fuzhou Maixin Biotech. Co., Ltd. Fuzhou, China). Diagnosis was based on positive staining by the HBC antibody. The reports of liver histology were generated by a pathologist. Liver function (shown in the serum ALT, AST TBIL) and kidney function (shown in serum creatinine, urea nitrogen) were both tested by the machine of Hitachi 7180 (Hitachi, Ltd., Tokyo, Japan). Normal range of ALT value is 5–35 IU/l. HBV DNA levels were measured by real-time fluorescence quantitative polymerase chain reaction assays (Applied Biosystems, ABI 7500, the lowest limit of detection was 100 IU/ml). HBsAg, HBeAg and anti-HBe were detected using Architect i2000SR (Abbott Laboratories). Hepatic inflammation was assessed using simple grading (score 1–4) and staging (score 0–4) systems for chronic viral hepatitis [20]. In brief, the different stages of chronic liver diseases relate to the degree of scarring, with the end stage being cirrhosis. The grade relates to the severity of the underlying disease process. The histology activity index (HAI) is appropriate for evaluation of a large numbers of patients.

Definitions

HBCag (−) or (+) was defined as the lack of or presence of HBCag, respectively, in the hepatocyte nucleus on IHC staining of liver biopsies (Fig. 2). Virological response was defined as undetectable serum HBV DNA (<2.0 log_{10} IU/ml) or undetectable with qPCR assay. HBeAg (−) was defined as <1 s/co. HBeAg seroconversion was defined as HBeAb >1 and HBeAg <1 s/co. The value of 1.00 to 3.00 in HBCAb was defined as negative, while 0 to 1.00 was defined as positive.

Statistical analyses

Appropriate statistical analysis was performed using Prism version 5 (GraphPad Software). Categorical variables were defined as proportion (%) and compared by chi-square or Fisher’s exact test. Continuous variables are depicted as mean ± standard deviation (SD) and were assessed by Student’s t-test or Mann–Whitney U test, as appropriate. Binary logistic regression analysis was performed in search of variables determining the HBeAg-negative status. Cumulative rates of complete viral suppression and ALT normalization were analysed by the Kaplan–Meier method. P < 0.05 was considered statistically significant.

Results

Patient characteristics

A total of 96 serum HBeAg (+) CHB patients were included in this study with 48 patients in the HBCag (−) group and 48 patients in the HBCag (+) group. They were all matched in age, sex, HBV DNA baseline, HBeAg levels, HBsAg levels, HBCab level and ALT levels (Table 1). The liver biopsy IHC stain for HBCag (−) and HBCag (+) is shown in Fig. 2A–E. There were no significant differences either in Knodell necroinflammatory grade or in cirrhosis stage (P = 0.280, 0.900, respectively.)
Virological response

Compared with patients in the HBcAg (+) group, the frequency of patients with undetectable HBV DNA in the HBcAg (-) group was significantly higher at week 48 (77.1% versus 45.8%, \( P = 0.002 \)) after starting of NA treatment and 96 (95.3% versus 83.3%, \( P = 0.045 \); Table 2). In addition, Kaplan–Meier survival analysis revealed that significant differences were found in HBV DNA levels between the two groups (\( P = 0.004 \)) by 288 weeks. The median survival time of HBV DNA was at 28.4 and 51.0 weeks (Fig. 3A).

ALT normalization rate

The ALT normalization rate progressively increased following administration of antiviral drugs treatment in both groups. ALT normalization rates were significantly higher in the HBcAg (-) group than in the HBcAg (+) group from week 48 to 144 (63.6% versus 43.7% at week 48, \( P = 0.041 \); 85.4% versus 62.5%, \( P = 0.011 \) at week 96; 93.7% versus 72.9% at week 144, \( P = 0.006 \), Table 2). However, based on the Kaplan–Meier survival analysis, it revealed that no significant difference was found in the cumulative ALT normalization rates (\( P = 0.058 \)) by 288 weeks and the median survival time of ALT normalization rates were 31.0 and 54.7 weeks (Fig. 3B).

HBeAg-negative rates

The frequency of HBeAg-negative expression was significantly higher in the HBcAg (-) group than in the HBcAg (+) group from week 96 to week 288 (35.4% versus 14.6% at week 96, \( P < 0.05 \); 60.4% versus 14.6%, \( P < 0.001 \) at week 144; 72.9% versus 35.4%, \( P < 0.001 \) at week 288; Table 2). In addition, Kaplan–Meier survival analysis revealed that the frequency of HBeAg-negative expression showed significant difference within the two groups (\( P < 0.001 \)) and the median survival time of HBeAg was 124 weeks in the HBcAg (-) group and 288 weeks in the HBcAg (+) group (Fig. 3).

HBeAg-negative rates in subgroup of histology

We grouped HBeAg (-) patients into subgroups according to histology grades (Grade 1 to Grade 3) and stages (Stage 1 to Stage 4).
Table 2 All correlated factors of HBeAg negative in the HBcAg(−) group and the HBcAg(+) group

| Items                        | HBcAg (−) (n = 48) | HBcAg (+) (n = 48) | P    |
|------------------------------|--------------------|--------------------|------|
| HBV DNA-negative rate (%)    |                    |                    |      |
| 24 weeks                    | 37.5 (18/48)       | 22.9 (11/48)       | 0.120|
| 48 weeks                    | 77.1 (37/48)       | 45.8 (22/48)       | 0.002*|
| 96 weeks                    | 95.3 (46/48)       | 83.3 (40/48)       | 0.045*|
| 144 weeks                   | 100 (48/48)        | 93.7 (45/48)       | 0.242|
| 288 weeks                   | 100 (48/48)        | 100 (48/48)        | 1.0  |
| ALT normalization rate (%)  |                    |                    |      |
| 24 weeks                    | 39.6 (19/48)       | 22.9 (11/48)       | 0.078|
| 48 weeks                    | 64.6 (31/48)       | 43.7 (21/48)       | 0.041*|
| 96 weeks                    | 85.4 (41/48)       | 62.5 (30/48)       | 0.011*|
| 144 weeks                   | 93.7 (45/48)       | 72.9 (35/48)       | 0.006*|
| 288 weeks                   | 95.8 (46/48)       | 89.6 (43/48)       | 0.435|
| HBeAg-negative rates (%)     |                    |                    |      |
| 24 weeks                    | 2.08 (1/48)        | 0/48               | 0.315|
| 48 weeks                    | 6.25 (3/48)        | 10.4 (5/48)        | 0.714|
| 96 weeks                    | 35.4 (17/48)       | 14.6 (7/48)        | 0.018*|
| 144 weeks                   | 60.4 (29/48)       | 20.8 (10/48)       | <0.001*|
| 288 weeks                   | 72.9 (35/48)       | 35.4 (17/48)       | <0.001*|
| HBeAg seroconversion rate at the end of follow-up (%) | 62.5 (30/48)       | 29.2 (14/48)       | 0.001*|
| HBeAg-negative rates in histology subgroup (%) |        |                    |      |
| Grade 1                     | 75.0 (3/4)         | 12.5 (1/8)         | 0.067|
| Grade 2                     | 72.4 (21/29)       | 38.7 (12/31)       | 0.011*|
| Grade 3                     | 73.3 (11/15)       | 44.4 (4/9)         | 0.212|
| HBeAg-negative rates in histology subgroup (%) |        |                    |      |
| Stage 0                     | 50.0 (4/8)         | 17.6 (3/17)        | 0.156|
| Stage 1                     | 87.5 (7/8)         | 71.4 (5/7)         | 0.569|
| Stage 2                     | 75.0 (6/8)         | 22.2 (2/9)         | 0.057|
| Stage 3                     | 71.4 (5/7)         | 44.4 (4/9)         | 0.358|
| Stage 4                     | 70.6 (12/17)       | 50.0 (3/6)         | 0.621|
| HBeAg-negative rates in subgroup of antiviral therapy (%) |        |                    |      |
| Total                       |                    |                    |      |
| LAM                         | 10 (10.4)          | 71.4 (5/7)         | 1.000|
| ADV                         | 11 (11.4)          | 71.4 (5/7)         | 1.000|
| LDT                         | 4 (4.2)            | 0 (0/2)            | 0.333|
| ETV                         | 54 (56.2)          | 78.3 (18/23)       | <0.001*|
There was a significant difference in the Grade 2 subgroup, with 72.4% (21/29) in HBcAg (−) group and 38.7% (12/31) in HBcAg (+) group ($P=0.011$). No significant difference was found in the Grade 1 or Grade 3 subgroups. There was also no significant difference within any of the stage subgroups (Table 2).

**HBeAg-negative rates in antiviral drug subgroups**

Six different antiviral drug therapies were used for patients in this study (LAM, ADV, LDT, ETV, LAM + ADV or ETV + ADV). The only significant difference in HBeAg-negative seroconversion was found in the ETV group. There was a higher frequency of HBeAg-negative seroconversion in the HBcAg (−) group than in the HBcAg (+) group (78.3%, 18/23 and 16.1%, 5/31, respectively), $P<0.001$ (Table 2).

**HBeAg (−) is a predictor of HBeAg seroconversion**

In the binary linear logistic regression analysis for all the factors in our study, we found that the absence of HBcAg was the only predictor for a patient being HBeAg (−) throughout the study. The Odds ratio (OR) value was 4.482 (95% CI 1.58–12.68) (Table 3).

**Discussion**

The strategy of finite-duration treatment with NAs can be feasible for HBeAg (+) patients who seroconvert to anti-HBe on treatment [22]. However, seroconversion rates in NA-naïve HBeAg (−) CHB patients undergoing NA treatment were low (LAM 50% [23], ADV 30–37% [24], LDT 53% [25] and ETV 26–49% [26]). In our study, about

---

**Table 2. Continued**

| Items                  | HBcAg (−) (n = 48) | HBcAg (+) (n = 48) | $P$ |
|------------------------|--------------------|--------------------|-----|
| LAM + ADV               | 14 (14.6)          | 87.5 (7/8)         | 0.245 |
| ETV + ADV               | 3 (3.1)            | 0 (0/1)            | 1.000 |

LAM: lamivudine; ADV: adefovir dipivoxil; LDT: telbivudine; ETV: entecavir; ALT: Alanine transaminase.

HBeAb reference: <1 s/co positive and >1 s/co negative.

*Significant difference was found in the HBcAg(−) group and HBcAg(+) group (All $P < 0.05$).
54.1% of all patients seroconverted, but 72.9% of HBcAg (−) patients became HBeAg (−) during the 288 weeks of NA therapy. These results show that HBcAg can serve as a predictor of virus response and HBeAg seroconversion.

The lack of HBcAg on IHC was a good predictor of viral response rates. We found that the viral response of the patient, as indicated by the loss of HBV DNA expression, in the HBcAg (−) group increased dramatically compared to the HBcAg (+) group from week 48 to week 96 ($P = 0.002$, $P = 0.045$, respectively). However, no difference was found between the two groups in week 144 (Table 2), suggesting that viral response rates were faster in the HBcAg (−) group. In addition, the frequency of HBV DNA-negative patients was significantly higher in the HBcAg (−) group than in the HBcAg (+) group. We conclude that the rates of viral response were faster in the HBcAg (−) group compared with the HBcAg (+) group. Therefore, the absence of HBcAg in the liver as determined by IHC could be a good predictor of viral response rates.

HBcAg (−) expression correlated with more significant ALT normalization rates. Normalization of ALT levels is used as a determinant of biochemical response [22]. ALT normalization rates were higher in the HBcAg (−) group than in the HBcAg (+) group from week 48 to week 144. However, there were no significant differences in cumulative ALT normalization rates in either group based on our study (Fig. 3). Therefore, these results indicate that a greater number of patients in the HBcAg (−) group recovered from liver injury and did so at a faster rate than the HBcAg (+) group. However, ALT activity often fluctuates over time and ALT normalization rates may be influenced by many other factors. Further multicenter research with more patients should be conducted to investigate the ALT normalization rates in the future.

The results of our study suggest that HBcAg (−) staining by IHC may be an important predictor of response to antiviral treatment, including HBeAg (−) rates. Our data indicated that HBeAg (−) rates were much higher in the HBcAg (−) group than the HBcAg (+) group from week 96 to week 288 (72.9% versus 35.4%, respectively, $P < 0.001$, Table 2). This was similar to HBeAg seroconversion rates at the end of the study (62.5% in HBcAg (−) versus 29.2% in HBcAg (+), $P = 0.001$). Although we separated HBeAg (−) patients from HBeAg (+) patients, we still found that the rates of HBcAg (−) patients were significantly different (Table 3).

### Table 3 The result of binary logistic lineal regression of the HBeAg negative in all patients

| Factors          | B     | S.E.  | Wald   | df  | Sig.  | Exp (B) | 95% CI for EXP (B) |
|------------------|-------|-------|--------|-----|-------|---------|-------------------|
|                  | Lower | Upper |        |     |       |         |                   |
| HBcAg (−)        | 1.500 | .531  | 7.987  | 1   | .005  | 4.482   | 1.584 12.683      |
| Sex (male)       | −.076 | .756  | .010   | 1   | .920  | .927    | .210 4.081        |
| Age              | −.294 | .335  | .769   | 1   | .380  | .745    | .386 1.438        |
| Antiviral drugs  |       |       |        | 5   | .182  |         |                   |
| LAM              | 1.741 | 1.585 | 1.206  | 1   | .272  | 5.703   | .255 127.459      |
| ADV              | 1.604 | 1.576 | 1.036  | 1   | .309  | 4.975   | .227 109.237      |
| LDT              | .463  | 1.362 | .116   | 1   | .734  | 1.589   | .110 22.914       |
| ETV              | .926  | 1.871 | .245   | 1   | .621  | 2.525   | .065 98.744       |
| LAM + ADV        | 2.507 | 1.563 | 2.573  | 1   | .109  | 12.271  | .573 262.613      |
| Histology grades (G) | .298 | .487  | .373   | 1   | .541  | 1.347   | .518 3.500        |
| Histology stages (S) | .216 | .197  | 1.201  | 1   | .273  | 1.241   | .843 1.827        |
| HBeAg baseline   | −.399 | .400  | .996   | 1   | .318  | .671    | .307 1.469        |
| HBsAg baseline   | −.304 | .457  | .443   | 1   | .506  | .738    | .301 1.806        |
| HBV DNA baseline | −.152 | .213  | .511   | 1   | .475  | .859    | .566 1.303        |
| ALT baseline     | .001  | .001  | 1.239  | 1   | .266  | 1.001   | .999 1.003        |
| HBcAb baseline   | 2.194 | 2.097 | 1.094  | 1   | .296  | 8.969   | .147 546.757      |
| Constant         | −2.172| 3.880 | .313   | 1   | .576  | .114    |                   |

*HBcAg negative was the only significant predictor.
Binary linear regression results also showed that a HBcAg (−) status was the very important predictor of a HBeAg (−) status, not including ETV or histology grade (Table 3). There are several reasons for how HBcAg (−) on IHC could predict the HBeAg (−) expression. First, HBcAg is one of the hepatitis B viral proteins [27] considered an indicator of active viral replication, especially as HBcAg expression patterns in hepatocytes had been found to be related to the activity of liver disease, hepatocyte proliferation and HBV DNA level [28]. As a result, a HBcAg (−) status means that there is less active viral replication and therefore lower propensity to make HBeAg. Second, HBcAg (−) status indicates a more active T cell response against HBV and thus results in less HBeAg expression. HBcAg expression in the nucleus is lower in patients with more active hepatitis B than in patients with inactive CHB [29, 30]. HBcAg can shift from the nucleus to the cytoplasm when cells undergo division after liver damage and HBcAg may be lower or lost in the nucleus with further reproduction of HBV DNA. Third, the presence of HBcAg in the hepatocyte nucleus may be positively correlated with intrahepatic cccDNA level. Lack of HBcAg in the hepatocyte indicates that the cccDNA could be very low or the supply for cccDNA pool could be deficient. Therefore, patients with a HBcAg (−) status during NA therapy may have better outcomes. This result was consistent with research by Uzun et al. [31], who also found that absence or a low level of HBcAg expression in the liver seemed to predict a patient’s response to antiviral treatment. Our results were also consistent with research by Lee et al. [14] that showed that patients with CHB with a HBcAg (−) status their hepatocyte nuclei had a better response to ETV. Taken together, we found that the absence of HBcAg in the hepatocyte nuclei on IHC could be a good predictor for HBeAg (−) expression in HBeAg (+) patients with CHB.

However, there are some limitations of this retrospective study. First, all the patients were enrolled at one institute, reducing the diversity of the population studied. Second, patients did not receive the same treatment of antiviral drugs, although most had ETV and even within this subgroup, we found an apparent difference between the two groups. We will continue to collect data from the ETV- and TDF-treated patients who are HBcAg (−). Finally, the genotype of HBV was not measured in the study. It has been reported that treatment responses rates to different NAs (including LAM, ADV, LDT and ETV) are similar in most HBV genotypes [32, 33]. In the future, to validate the value of the HBcAg expression status in hepatocytes for predicting response to NAs, further research on the mechanisms with which HBcAg is involved in the hepatocyte nucleus is needed. However, based on our rigorous analysis and investigation, we strongly believe that HBcAg (−) status on liver biopsy can be considered a good predictor for HBeAg seroconversion rates in HBeAg (+) patients with CHB.

In conclusion, lack of HBcAg in the hepatocyte nucleus on IHC of a liver biopsy could be an independent predictor for likelihood of HBeAg seroconversion during in NA-naïve patients with CHB who are HBeAg (+). The function and underlying mechanisms of the HBcAg in the hepatocyte nucleus during NA therapy in patients with CHB need to be further investigated.

**Acknowledgements**

We would like to thank all study subjects who volunteered their participation in this study.

**Funding source:** This project was supported by Guangdong Science and Technology Plan Projects (No. 2014A020212478) and the Natural Science Foundation of China (No. 81501744).

**Conflict of interest**

The authors declare that they have no competing interests.

**Author contributions**

J.X., X.L., M.H. conceptualized the study. M.H. and J.L. conducted the data analysis, interpreted results and wrote the manuscript. M.C., X.Z., Z.H., Z.H., J.X. and Z.Z. performed the fieldwork and data collection. M.H. and J.L. performed the data management. X.L. and J.X. contributed to interpretation of the results and the manuscript writing. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

This study was approved by the 5th Affiliated Hospital Ethical Committee at SYSU. Written consent to participate in the study was obtained from each participant in the study.

**References**

1. Ott JJ, Stevens GA, Groeger J, et al. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine. 2012; 30: 2212–9.

2. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015; 385: 117–71.

3. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006; 295: 65–73.

4. Yuen MF, Hou JL, Chutaputti A. Asia Pacific working party on prevention of hepatocellular carcinoma. J Gastroenterol Hepatol. 2009; 24: 346–53.

5. Raimondo G, Recchia S, Lavarini C, et al. Dane particle-associated hepatitis B e antigen in patients with chronic hepatitis B virus infection and hepatitis...
B e antibody, Hepatology. 1982; 2: 449–54.
6. Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med. 2002; 347: 168–74.
7. Lin SM, Sheen IS, Chien RN, et al. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology. 1999; 29: 971–5.
8. Farrell G. Hepatitis B e antigen seroconversion: effects of lamivudine alone or in combination with interferon alpha. J Med Virol. 2000; 61: 374–9.
9. Zoutendijk R, Reijnders JG, Brown A, et al. Entecavir treatment for chronic hepatitis B: adaptation is not needed for the majority of naïve patients with a partial virological response. Hepatology. 2011; 54: 443–51.
10. Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet. 2013; 381: 468–75.
11. Lok AS, Trinh H, Carosi G, et al. Efficacy of entecavir or without tenofovir disoproxil fumarate for nucleos(t)ide-naïve patients with chronic hepatitis B. Gastroenterology. 2012; 143: 619–28.
12. Hsu HC, Su IJ, Lai MY, et al. Biologic and prognostic significance of hepatocyte hepatitis B core antigen expressions in the natural course of chronic hepatitis B virus infection. J Hepatol. 1987; 5: 45–50.
13. Thimme R, Wieland S, Steiger C, et al. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol. 2003; 77: 68–76.
14. Lee JG, Hwang SG, Yoon H, et al. Hepatitis B core antigen expression in hepatocytes reflects viral response to entecavir in chronic hepatitis B patients. Gut Liv. 2013; 7: 462–8.
15. Hadziyannis SJ, Lieberman HM, Karvountzis GG, et al. Analysis of liver disease, nuclear HBcAg, viral replication, and hepatitis B virus DNA in liver and serum of HBsAg vs. anti-HBe positive carriers of hepatitis B virus. Hepatology. 1983; 3: 656–62.
16. Serinoz V, Varli M, Erden E, et al. Nuclear localization of hepatitis B core antigen and its relations to liver injury, hepatocyte proliferation, and viral load. J Clin Gastroenterol. 2003; 36: 269–72.
17. Krivadis A, Kotoula V, Soultouvannis I, et al. Detectable HBV cccDNA in liver biopsy specimens correlates with the expression of HBcAg in chronic hepatitis B. J Hepatol. 2005; 42: S2–171.
18. Nossal MB. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. Gut. 2015; 64: 1972–84.
19. Von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007; 370: 1453–7.
20. Goodman ZD. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol. 2007; 47: 598–607.
21. Chinese Society of Hepatology and Chinese Society of Infectious Diseases CMA. The guideline of prevention and treatment for chronic hepatitis B (2010 version). Zhonghua Gan Zang Bing Za Zhi. 2011; 32: 405–15.
22. Sarin SK, Kumar M, Lai GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016; 10: 1–98.
23. Yao GB, Zhu M, Cui AY. A 7-year study of lamivudine therapy for chronic hepatitis B virus e antigen-positive chronic hepatitis B patients in China. J Dig Dis. 2009; 10: 131–7.
24. Zeng M, Mao Y, Yao GB, et al. Five years of treatment with adefovir dipivoxil in Chinese patients with HBeAg-positive chronic hepatitis B. Liver Int. 2012; 32: 137–46.
25. Wang Y, Thongsawat S, Gane EJ, et al. Efficacy and safety of continuous 4-year telbivudine treatment in patients with chronic hepatitis B. J Viral Hepat. 2013; 20: e37–46.
26. Wong GL, Wong VW, Chan HY, et al. Undetectable HBV DNA at month 12 of entecavir treatment predicts maintained viral suppression and HBeAg-seroconversion in chronic hepatitis B patients at 3 years. Aliment Pharmacol Ther. 2012; 35: 1326–35.
27. Cao T, Meuleman P, Desombere I, et al. Leroux-Roels G In vivo inhibition of anti-hepatitis B virus core antigen (HBcAg) immunoglobin G production by HBeAg-specific CD4(+)/Th1-type T-cell clones in a hu-PBL-NOD/SCID mouse model. J Virol. 2001; 75: 11449–56.
28. Son MS, Yoo JH, Kwon CI, et al. Associations of expressions of HBcAg and HbsAg with the histologic activity of liver disease and viral replication. Gut Liv. 2008; 2: 166–73.
29. Chu CM, Yeh CT, Sheen IS, et al. Subcellular localization of hepatitis B core antigen in relation to hepatocyte regeneration in chronic hepatitis B. Gastroenterology. 1995; 109: 1926–32.
30. Sansonno DE, Fiore G, Buano G, et al. Cytoplasmic localization of hepatitis B core antigen in hepatitis B virus infected livers. J Immunol Methods. 1988; 109: 245–52.
31. Uzun Y, Bozkaya H, Erden E, et al. Hepatitis B core antigen expression pattern reflects the response to anti-viral treatment. J Gastroenterol Hepatol. 2006; 21: 977–81.
32. Liu CJ, Kao JH, Chen DS. Therapeutic implications of hepatitis B virus genotypes. Liver Int. 2005; 25: 1097–107.
33. Wiegand J, Hasenclever D, Tillmann HL. Should treatment of hepatitis B depend on hepatitis B virus genotypes? A hypothesis generated from an explorative analysis of published evidence. Antivir Ther. 2008; 13: 211–20.