A large population study of spontaneous HBeAg seroconversion and acute exacerbation of chronic hepatitis B infection: implications for antiviral therapy

M-F Yuen, H-J Yuan, C-K Hui, D K-H Wong, W-M Wong, A O-O Chan, B C-Y Wong, C-L Lai

Background and aim: Clinical data on spontaneous hepatitis B e antigen (HBeAg) seroconversion and acute exacerbation of chronic hepatitis B (CHB) virus infection from large population studies are lacking. In the present study we examined the clinical features and significance of HBeAg seroconversion and acute exacerbation in 3063 Chinese CHB patients.

Methods: Clinical assessment, liver biochemistry, hepatitis B virus (HBV) serology and HBV DNA, time of HBeAg seroconversion, and acute exacerbation were monitored.

Results: Median age at HBeAg seroconversion was 34.5 years. The cumulative HBeAg seroconversion rate significantly increased with alanine aminotransferase (ALT) levels on presentation (p<0.0001). For patients with ALT levels more than twice the upper limit of normal (ULN) on presentation, the HBeAg seroconversion rate at the fifth year of follow up was 72.4%. After HBeAg seroconversion, 65.2% [73/110] of patients had undetectable HBV DNA levels by the Digene Hybrid Capture assay. Of these, 78.1% still had HBV DNA levels detectable by the Amplicor HBV Monitor Test. We found that 37.5% antibody to HBeAg (anti-HBe) positive patients had undetectable HBV DNA levels by the Digene Hybrid Capture assay before acute exacerbation. Acute exacerbations of longer duration, with higher peak ALT, bilirubin, and α-fetoprotein levels were associated with an increased HBeAg seroconversion rate (p<0.0001–0.045). Acute exacerbation with peak ALT levels more than five times the ULN carried a 46.4% chance of HBeAg seroconversion within three months. HBeAg seroconversion and mortality occurred in 2.7% and 0.7% of acute exacerbations, respectively.

Conclusion: In the present study we have provided information on HBeAg seroconversion and acute exacerbation, which are important in decision making for CHB treatment and in designing clinical trials.

PATIENTS AND METHODS
From 1976 to 2000, 3063 patients attending the Hepatitis Clinic, Queen Mary Hospital, University of Hong Kong, Hong Kong, were recruited into the present study. Patients were all hepatitis B surface antigen (HBsAg) positive for at least six months. Patients were excluded from the study if they had any of the following conditions: (1) cirrhosis related complications or HCC on presentation, (2) treatment for CHB at any time, (3) concomitant hepatitis C or D virus infection, (4) evidence of autoimmune hepatitis, Wilson's disease, or primary biliary cirrhosis, (5) history of heavy alcoholic intake, (6) evidence of fatty liver, and (7) intake of any hepatotoxic drugs. Patients had complete history enquiry and physical examination during the first clinic visit. Blood was taken for liver biochemistry, α-fetoprotein (AFP), and HBV serological markers, including HBsAg, HBeAg, and anti-HBe by microparticle enzyme immunoassay (MEIA; Abbott Laboratories, Chicago, Illinois, USA) during the first visit and every subsequent visit which was scheduled at intervals of 3–6 months, or more frequently whenever indicated.

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Abbreviations: CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; anti-HBe, antibody to HBeAg, HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; HBV, hepatitis B virus; AFP, α-fetoprotein; ULN, upper limit of normal; PCR, polymerase chain reaction.

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ups. In patients who had HBeAg seroconversion, HBeAg sero-reversion was defined as subsequent loss of anti-HBe and regaining of HBeAg on at least two consecutive follow ups.

Acute HBV exacerbation was defined as an increase in ALT to more than 1.5 times the upper limit of normal (ULN) after excluding other common causes of ALT elevation, including other viral hepatitis, drug induced hepatitis, alcoholic hepatitis, and steatohepatitis. Time, duration, peak ALT level, peak AFP, peak bilirubin level, and HBeAg-anti-HBe status were all monitored during HBV exacerbation. HBeAg seroconversion was regarded as related to acute exacerbation if HBeAg seroconversion occurred within three months of acute exacerbation.

HBV DNA levels before and after HBeAg seroconversion were measured in 110 patients by Digene Hybrid Capture assay (Digene Corporation, Gaithersburg, Maryland, USA) (lower limit of detection 140 000 copies/ml). For those with undetectable HBV DNA levels by the above assay, HBV DNA levels were determined again by a more sensitive quantitative polymerase chain reaction (PCR) assay (Cobas Amplicor HBV Monitor Test; Roche Diagnostics, Branchburg, New Jersey, USA) (lower limit of detection 200 copies/ml).

**Statistical analysis**

All statistical analyses were performed using the Statistical Program for Social Sciences (SPSS 10.0 for Windows, SPSS Inc., Chicago, Illinois, USA). Data were analysed by Mann-Whitney test for continuous ordinal data. Differences in paired parameters were tested by the Wilcoxon signed ranks test. The Kaplan-Meier method was applied for calculation of the cumulative rate of HBeAg seroconversion and the cumulative HBeAg seroconversion rate was studied by the log rank test. A p value of less than 0.05 was considered statistically significant.

**RESULTS**

Demographic data on presentation for the 3063 patients are listed in table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0005</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>0.016</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3</td>
<td>0.003</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4</td>
<td>0.003</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>5</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Of the 1215 patients who were HBeAg positive on presentation, 493 (40.6%) underwent HBeAg seroconversion during the follow up period. Median age at HBeAg seroconversion was 34.5 years (range 3.6–77.4). There was no difference in the cumulative HBeAg seroconversion rate between male and female patients (p=0.53).

The cumulative HBeAg seroconversion rate was studied by stratifying patients according to ALT levels on presentation: group 1, patients had ALT levels <0.5×ULN; group 2 patients had ALT levels >0.5–1×ULN; group 3 patients had ALT levels >1–2×ULN; group 4 patients had ALT levels >2–5×ULN; and group 5 patients had ALT levels >5×ULN. Higher ALT levels on presentation were associated with a higher chance of HBeAg seroconversion (overall p<0.0001) (fig 1). Cumulative HBeAg seroconversion rates according to the different ALT levels on presentation are listed in table 2.

In 110 patients with paired sera before and after HBeAg seroconversion, median HBV DNA level was significantly lower after HBeAg seroconversion than before HBeAg seroconversion (<0.142 (range <0.142–309.5×10<sup>3</sup>) vs 4.0) (range <0.142–803.9 ×10<sup>3</sup>) copies/ml; p<0.0001). Seventy three patients (65.2%) had undetectable HBV DNA by the Digene Hybrid Capture assay after HBeAg seroconversion. However, when sera of these 73 patients were retested using the Cobas AmpliC/tr HBV Monitor Test, only 16 (21.9%) had undetectable HBV DNA. The median HBV DNA level of the remaining 57 patients was 3150 copies/ml (range 200–327000 000). HBV DNA levels were in the range 200–10<sup>3</sup>, >10<sup>3</sup>–10<sup>4</sup>, and >10<sup>4</sup> in 11 (15.1%), 17 (23.3%), 25 (34.2%), and 4 (5.5%) patients, respectively.

A total of 1670 patients (54.5%) had ALT levels less than 1.5×ULN throughout the whole follow up period—that is, more than half of the Chinese CHB patients had near normal ALT levels over a prolonged period of time. The remaining 739 patients (45.5%) (765 HBeAg positive, 628 anti-HBe positive) had at least one episode of acute exacerbation (929 on presentation and 464 on subsequent follow up). Only 204 patients (14.6%) experienced hepatitis symptoms. Excluding the 929 patients with acute exacerbation on presentation, the cumulative risk of acute exacerbation during follow up is illustrated in fig 2.

Of the 765 HBeAg positive patients with acute exacerbation, 297 patients (38.8%) had HBeAg seroconversion within three months of acute exacerbation. The differences in the factors of acute exacerbation between patients with and without HBeAg seroconversion are listed in table 3. (For patients who presented with acute exacerbation, the earliest parameters were taken as peak values if subsequent values were not increasing.) HBeAg seroconversion occurred in 17 of 628 anti-HBe positive patients (2.7%) after acute exacerbation. There were no differences in median peak ALT levels, peak bilirubin levels, peak AFP levels, and duration of acute exacerbation between anti-HBe positive patients with and without HBeAg seroconversion. Ten patients (0.7%) died from acute exacerbation due to hepatic decompensation.
within three months of acute exacerbation in patients with peak AFP levels of <20, >20–100, and >100 ng/ml were 35.2%, 43.6%, and 50.5%, respectively. Of 336 patients with peak ALT levels >5×ULN and 91 patients with peak AFP levels >100 ng/ml, 70 patients had both of the above parameters. Forty of these 70 patients (57.1%) had HBeAg seroconversion within three months of acute exacerbation.

Compared with anti-HBe positive patients, HBeAg positive patients had significantly higher median peak ALT levels (148 (range 75–8000) U/l, respectively; p<0.0001), and longer median duration of acute exacerbation (17 (range 3–416) v 21 (2–436) weeks, respectively; p=0.011).

HBV DNA levels within three months prior to acute exacerbation were measured in 46 HBeAg positive patients (14 with HBeAg seroconversion and 32 without HBeAg seroconversion after acute exacerbation) and 32 anti-HBe positive patients. In HBeAg positive patients, there was a trend for patients with HBeAg seroconversion to have lower HBV DNA levels before acute exacerbation compared with patients who did not achieve HBeAg seroconversion (29.0 (range 0.26–817.7×10⁶) v 241.8 (range 0.27–1700×10⁶) copies/ml; p=0.073). The median HBV DNA level was significantly higher in HBeAg positive patients compared with that of anti-HBe positive patients (182.2 (range 0.26–1700) v 0.31 (<0.142–407.0)×10⁶ copies/ml, respectively; p<0.0001). All 46 HBeAg positive patients had detectable HBV DNA levels above 0.142×10⁶ copies/ml (lower limit of detection of Digene Hybrid Capture assay) whereas 12 anti-HBe positive patients (37.5%) had HBV DNA levels below 0.142×10⁶ copies/ml within three months prior to acute exacerbation. Of these 12 patients, 11 (91.7%) had detectable HBV DNA, ranging from 537 to 45 400 copies/ml, and one patient (8.3%) had undetectable HBV DNA by the Amplicor HBV Monitor Test.

**DISCUSSION**

This large population study has provided essential information on the probability of HBeAg seroconversion when assessing patients on presentation. The overall HBeAg seroconversion rate was 18% and 53.5% for the first and fifth years, respectively (table 2). In general, the higher the ALT level on presentation, the higher the chance of HBeAg seroconversion during subsequent follow up. Table 2 provides a guide for the design of therapeutic trials whereby one can balance the chance of spontaneous HBeAg seroconversion and the chance of HBeAg seroconversion induced by treatment.

Even when mild elevation of ALT levels to above 1.5×ULN was taken as acute exacerbation, 54% of patients still did not have any exacerbation throughout their follow up period. Therefore, unlike in Caucasian CHB patients, Chinese CHB patients often had normal or near normal ALT levels. Various factors during acute exacerbation were identified as predisposing patients to HBeAg seroconversion. Patients with peak ALT levels > 5×ULN and peak AFP levels >100 ng/ml had a 57.1% chance of HBeAg seroconversion within three months of occurrence of acute exacerbation. These two parameters may reflect, respectively, the degree of immune mediated attack on infected hepatocytes and hepatocyte regeneration afterwards. Additional information that has not been well studied in the past was duration of acute exacerbation.

**Table 2** Cumulative hepatitis B e antigen (HBeAg) seroconversion rates in patients with different alanine aminotransferase levels on presentation

<table>
<thead>
<tr>
<th>Cumulative HBeAg seroconversion rate (%)</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
<th>Year 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (n=1215)</td>
<td>18.0</td>
<td>31.0</td>
<td>41.3</td>
<td>47.6</td>
<td>53.5</td>
</tr>
<tr>
<td>Stratified by ALT levels on presentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT &lt;0.5×ULN (n=151)</td>
<td>9.4</td>
<td>16.0</td>
<td>19.9</td>
<td>23.0</td>
<td>26.5</td>
</tr>
<tr>
<td>ALT &gt;0.5–1×ULN (n=294)</td>
<td>11.7</td>
<td>22.6</td>
<td>32.3</td>
<td>39.5</td>
<td>47.9</td>
</tr>
<tr>
<td>ALT &gt;1–2×ULN (n=284)</td>
<td>12.8</td>
<td>28.9</td>
<td>40.1</td>
<td>46.2</td>
<td>53.0</td>
</tr>
<tr>
<td>ALT &gt;2–5×ULN (n=486)</td>
<td>28.2</td>
<td>44.9</td>
<td>58.4</td>
<td>66.6</td>
<td>72.4</td>
</tr>
<tr>
<td>ALT &gt;5×ULN (n=243)</td>
<td>24.9</td>
<td>39.3</td>
<td>53.5</td>
<td>59.8</td>
<td>67.5</td>
</tr>
<tr>
<td>ALT &gt;5×ULN (n=243)</td>
<td>31.5</td>
<td>50.6</td>
<td>63.7</td>
<td>74.7</td>
<td>78.1</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; ULN, upper limit of normal.

**Table 3** Parameters of acute exacerbation in patients with and without hepatitis B e antigen (HBeAg) seroconversion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acute exacerbation with HBeAg seroconversion (n=297)</th>
<th>Acute exacerbation without HBeAg seroconversion (n=468)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak ALT (U/l)</td>
<td>279 (75–3990)</td>
<td>191 (75–3955)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peak bilirubin (µmol/l)</td>
<td>17 (4–390)</td>
<td>15 (2–868)</td>
<td>0.045</td>
</tr>
<tr>
<td>Peak AFP (ng/ml)</td>
<td>10 (1–3958)</td>
<td>7 (1–2013)</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of acute exacerbation (weeks)</td>
<td>24 (1–236)</td>
<td>17 (1–436)</td>
<td>0.018</td>
</tr>
</tbody>
</table>

All values are median (range).

ALT, alanine aminotransferase; AFP, α fetoprotein.
found that patients with a longer duration of acute exacerbation had a higher chance of HBeAg seroconversion subsequently (table 3).

Mortality due to acute exacerbation was uncommon, occurring in only 0.7% of patients. These data do not suggest that treatment with lamivudine or interferon-alpha for patients with acute HBV exacerbation is indicated routinely. In fact, the high rate of spontaneous HBeAg seroconversion should be borne in mind when one is considering initiation of treatment with one of these agents. In case of high ALT levels, prophylactic treatment is indicated routinely. In fact, the high rate of spontaneous HBeAg seroconversion should be borne in mind when one is considering initiation of treatment with one of these agents.

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REFERENCES