Spontaneous Seroconversion in Hepatitis B e Antigen–Positive Chronic Hepatitis B: Implications for Interferon Therapy

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This study compared rates of spontaneous hepatitis B e antigen (HBeAg)–positive to –negative seroconversion in chronic carriers of hepatitis B virus (HBV) with rates reported during interferon-α therapy. Four hundred fifty-four Asian-American HBeAg-positive HBV carriers, followed for 1–10 years, were tested approximately every 6 months for HBeAg. Patients with alanine aminotransferase levels ≥50 IU/mL at entry had 1067.3 seroconversions/10^5 person-months in the 5– to 19-year age group, 1753.3 in the 20– to 34-year group, and 1257.2 in the 35– to 50-year group. Published data indicate that 30% of children and 33% of adults seroconvert during interferon-α treatment and follow-up. In our study population, spontaneous seroconversion occurred in 15% of children (95% confidence interval [CI], 8%–27%), 23% of adults 20–34 years (95% CI, 15%–34%), and 17% of adults 35–50 years (95% CI, 10%–28%) during the same interval. The high rate of spontaneous seroconversion should be weighed in decisions to treat HBV carriers with interferon-α.

Chronic hepatitis B virus (HBV) infection exhibits a wide spectrum of clinical manifestations, from a completely asymptomatic condition detectable only by the long-term presence of serum hepatitis B surface antigen (HBsAg) to severe liver disease resulting in cirrhosis or hepatocellular carcinoma (HCC) [1]. Among chronically infected persons, the presence of serum hepatitis B e antigen (HBeAg) indicates active viral replication, higher infectivity, and the potential for more liver damage [1, 2]. HBeAg appears early in the course of infection and often persists in chronic infection. Development of antibody to HBeAg (anti-HBe) usually follows seroconversion from HBeAg-positive to HBeAg-negative.

The aim of the present study was to estimate the spontaneous HBeAg-positive to -negative seroconversion rate in a population of Asian-American HBV carriers identified through community screening and to assess the benefit these patients might derive from interferon-α therapy. Long-term prospective studies of the natural history of the HBeAg-positive carrier state are rare [3]. Much of what is known about the natural course of HBeAg-positive hepatitis has been derived from studies of patients in the tertiary care setting who presented with a liver-related complaint. Among chronic carriers identified incidentally (as in blood donation), the prevalence of HBeAg is usually low [4].

Randomized controlled trials of interferon-α for HBeAg-positive chronic hepatitis have shown that treatment can increase the probability of seroconversion to HBeAg-negative. Nonetheless, treatment with interferon-α is ineffective for one-half to two-thirds of patients. In a metaanalysis of published clinical trials, Wong et al. [5] estimated that 33% of treated subjects seroconverted to HBeAg-negative compared with 12% of untreated controls during follow-up periods of 12–18 months. Treatment of healthy HBeAg-positive carriers is not recommended [6, 7], and even among HBeAg-positive patients eligible for treatment, it is those with the highest elevations of alanine aminotransferase (ALT) level or histologic evidence of liver damage that are most likely to respond [6, 8]. The information provided by this study may be useful to clinicians counseling patients with HBeAg-positive hepatitis.

Methods

Study population. Since 1985, the Liver Cancer Prevention Center at Fox Chase Cancer Center has followed 1522 Asian-American HBV carriers by use of serologic tests for viral and biochemical markers of liver disease. The population includes both immigrants and persons born in the United States. Nearly all patients were initially identified as HBsAg-positive through screening campaigns in ethnic Asian communities in the Philadelphia area and were requested to return for serologic tests either every 6 months or every year (for carriers <17 years) regardless of HBeAg status. From an initial population of 2599 carriers identified through screening, 1522 chose to return for at least one follow-up visit. Among these, 524 (34.4%) were HBeAg-positive on at least one occasion. The primary purpose of the follow-up procedure was the early detection of HCC [9].

For this analysis, subjects aged 5–50 years who were HBeAg-positive at least once during a minimum of 6 months of follow-up and from whom at least 2 blood samples were collected were included. None of the patients received immunosuppressive or interferon-α therapy during the study period, and 5 patients positive for antibody to the hepatitis C virus were excluded. Four hundred fifty-four patients met these criteria. The median follow-up time was 25 months (range, 6–154). The median number of
samples per subject was 4 (range, 2–34). One subject was known
to have died during the follow-up period. The cause of death was
HCC, and this subject had not seroconverted from HBeAg prior
to diagnosis. Compared with 789 HBeAg-positive subjects in the
same age range at study entry who did not return for follow-up
after initial screening, the subjects included in this analysis were
significantly younger (mean age ± SD, 21.9 ± 11.3 vs. 26.8 ±
11.4; \( P < .001 \)) and more likely to have ALT levels >50 IU/mL
at study entry (21.4% vs. 10.3%, \( P < .001 \)).

Among the 932 subjects aged 5–50 years who were HBeAg-
negative at study entry and who were followed longitudinally from
the original cohort (not included in this analysis), 44 (4.7%) re-
verted to HBeAg-positive at least once during follow-up. Five of
932 subjects were known to have died during follow-up. Three
deaths were from HCC (1 present at study entry) and 2 from other
causes.

Serologic testing. At each visit, serum samples from subjects
were tested for markers of HBV infection (HBsAg, antibody to
HBsAg, HBeAg, anti-HBe, HBV DNA) and other biochemical
markers of liver disease (ALT, alphafetoprotein, ferritin). HBV
DNA was first assayed by dot blot and, if negative by dot blot,
by Southern hybridization using a \(^{3}P\)-labeled RNA complemen-
tary to HBV minus-strand DNA. The sensitivity of the Southern
blot was \(~0.1 \mu g of HBV DNA or \sim10^6\) HBV particles/mL. All
other HBV markers were tested by EIA using commercial kits
(Abbott Laboratories, Abbott Park, IL).

Statistical analysis. Survival and Poisson regression analyses
were done using EGRET (Statistics and Epidemiology Research
Corporation, Seattle). Variable age at study entry was accounted
for by left truncation \[10\]. Time of seroconversion was estimated
as the midpoint between the last HBeAg-positive blood test and
the first HBeAg-negative test. Poisson regression was used to pro-
duce the age-specific incidence rates \[11\].

We compared seroconversion rates in our study population with
those reported in the literature for interferon-\(\alpha\) clinical trials in
children and adults. For children, we used the median response
rate of 30% from three published randomized, controlled trials
\[12–14\]. One published trial involving children \[15\] was excluded
because all subjects had normal serum ALT levels. For adults,
we used the overall response rate of 33% from the metaanalysis of
randomized, controlled interferon-\(\alpha\) trials by Wong et al. \[5\].
We used the median duration of therapy plus follow-up from the adult
trials—15 months—as the reference time period. It is appropri-
tate to include the follow-up time in this comparison because some
patients who seroconvert to HBeAg-negative do so after treatment
has ended and because some patients who initially seroconvert
while receiving interferon will revert to HBeAg-positive during
the follow-up period. Details of the methods used to calculate the
comparison measures are given in the Appendix.

Results

Four hundred fifty-four HBeAg-positive HBV carriers were
followed for 17,787 person-months. Characteristics at study
entry are summarized in table 1. Country of birth was recorded
for 342 subjects (75.3%). Of these, 326 (95.3%) were born
outside the United States. Of 435 tested for HBV DNA at study
entry, 420 (96.6%) were positive. The serum ALT level was
initially elevated (>50 IU/mL) in 96 (21.4%) of 448 carriers
for whom measurements were available; 22 (22.9%) were >2.5
times the upper limit of normal (>125 IU/mL).

During follow-up, 108 subjects (23.8%) seroconverted to
HBsAg-negative. Subjects who were HBeAg-negative for at
least two consecutive blood tests at least 6 months apart were
considered to have undergone stable seroconversion. Thirty-
eight (35.2%) seroconversions could not be classified as either
stable or unstable due to insufficient follow-up. Of 70 sero-
conversions with sufficient follow-up, 58 (82.9%) were classified
as stable. Seven of these (12.1%) had at least one reversion
to HBeAg-positive before becoming stably HBeAg-negative.
Twelve (17.1%) of the 70 seroconversions with adequate fol-
low-up time remained HBeAg-negative for <6 months after
seroconversion and/or had reverted to HBeAg-positive at last
follow-up visit and were considered unstable seroconversions for
this analysis. These patients tended to have multiple
HBeAg-positive to HBeAg-negative seroconversions of <6
months duration. At the time of first seroconversion to HBeAg-
negative, 44 (75.9%) of the stable group, 5 (41.7%) of the
unstable group, and 27 (71.1%) of the group with inadequate
follow-up were positive for anti-HBe. The group with inade-
quate follow-up was not statistically significantly different from
the group with stable seroconversions in age, sex, or ALT
level at study entry. The outcomes of the 454 subjects are
summarized in figure 1.

Of 58 patients who had stable seroconversions, 54 (93.1%)
had serum HBV DNA detectable by dot blot or Southern blot
prior to seroconversion. At their latest tests, the 4 subjects who
were HBV DNA-negative prior to seroconversion remained
HBV DNA-negative. Thirty-nine (72.2%) of the 54 patients
who were HBV DNA-positive before seroconversion have ex-
perienced occasional brief reversion to HBV DNA-positive
during follow-up after becoming HBV DNA-negative. At last
follow-up, 49 (84.5%) of the 58 patients were HBV DNA-
negative. Two subjects among those who had stable sero-
conversions became HBsAg-negative after HBeAg serocon-
version, 1 at 5 years later and the other at 9 years later.

| Table 1. Characteristics of chronic carriers of HBV at study entry (n = 454). |
|-------------------------|-----------------|-----------------|--------|
| Age group               | Mean age (±SD)  |                   |
| 5–19 years              | 47.4 (215)      |                   |
| 20–34 years             | 38.5 (175)      |                   |
| 35–49 years             | 14.1 (64)       |                   |
| Male                    | 61.5 (279)      |                   |
| Ethnic group            |                  |                   |
| Southeast Asian*        | 47.4 (215)      |                   |
| Chinese                 | 29.1 (132)      |                   |
| Korean                  | 23.3 (106)      |                   |
| Japanese                | 0.2 (1)         |                   |

NOTE. Except for age, data are % (no.).
* Vietnamese, Laotian, Cambodian, Hmong, or Thai.
Figure 1. Follow-up diagram for 454 patients positive for hepatitis B e antigen (HBeAg) at study entry. ALT, alanine aminotransferase; (−), negative.

Thirty-nine (67.2%) of 58 persons who stably seroconverted had elevated ALT levels at least once before seroconversion, and 15 (25.7%) had levels >2.5 times the upper limit of normal. Fifty-two (89.7%) of 58 subjects had normal ALT levels by the second visit following seroconversion.

Figure 2 shows the product-limit estimates of the proportion of subjects who stably seroconverted to HBeAg-negative by age. The analysis shows that among subjects HBeAg-positive at age 5, 25% had a stable seroconversion by age 17, 50% by age 24, and 75% by age 33. At age 50, 8% remained HBeAg-positive. After age 50, seroconversions were rare (data not shown). There was no significant difference in the incidence of stable seroconversion between female and male carriers (relative risk [RR] = 0.88; 95% confidence interval [CI], 0.50–1.6) controlling for ALT status. Subjects who had an elevated ALT level at study entry were significantly more likely to seroconvert during follow-up (RR = 3.3; 95% CI, 1.9–5.8) controlling for gender.

The incidence rates of stable seroconversion per person-month were estimated by age group and initial ALT status. Subjects included in this analysis were those with stable seroconversions or at least 1 year of follow-up from study entry or both (n = 338). This restriction was made because a mini-
mum of 1 year of observation is required to observe a seroconversion and determine stability 6 months later. Subjects who had unstable seroconversions were included and treated as censored at the time of their first seroconversion. There were 12,388 person-months of follow-up. The model included age group, ALT status, and an interaction term. Although the interaction term was not statistically significant \((P = .07)\), it was retained in the model to preserve the difference in the change in seroconversion rates with age in the normal versus elevated ALT groups. Table 2 shows the estimated incidence rates.

A second model was fit using data only from subjects who had an elevated ALT level at any time prior to seroconversion \((n = 118)\). Person-time was included from the time of the first elevated ALT level rather than from study entry. There were 39 stable seroconversions in 2812 person-months of observation. The last column of table 2 shows the estimated incidence rates of stable seroconversion for this model.

In order to provide incidence rates of seroconversion among patients as comparable as possible to those meeting eligibility criteria for most interferon clinical trials, we also calculated the rates by age group for subjects with elevated ALT level who were HBeAg-positive for at least 6 months and 2 consecutive blood samples. There were 25 stable seroconversions in 2266 person-months of observation in this group. The incidence rates per 100,000 person-months were 1071.6 (95% CI, 481.7–2384.2) for the 5–19 year age group, 1301.8 (95% CI, 700.5–2419.4) for the 20–34 year age group, and 958.6 (95% CI, 498.8–1842.3) for the 35–50 year group.

Comparison with interferon-\(\alpha\) therapy. Table 3 shows the comparison of spontaneous versus interferon-\(\alpha\) seroconversion rates for patients with elevated ALT levels. We estimated the cumulative incidence of seroconversion within 15 months for each age group in the untreated study patients versus response percentages published from randomized clinical trials. The seroconversion rate predicted for interferon-\(\alpha\) therapy was modestly higher in each age group, and the difference from the probability of spontaneous seroconversion in the same time period was 10%–16%. Because spontaneous seroconversion is an ongoing process, we also estimated the additional time (>15 months) necessary for spontaneous seroconversions to equal the probability of seroconversion reported for interferon-\(\alpha\) therapy. These differences ranged from 8 to 18 months of additional time needed to achieve an equal probability of seroconversion.

### Discussion

Interferon-\(\alpha\) is the only drug currently approved for the treatment of chronic HBV infection. For treated patients who seroconvert to HBeAg-negative, long-term prognosis appears to be good [16, 17]. Most patients treated with interferon-\(\alpha\), however, do not seroconvert. The long-term effects of treatment on nonresponders are more difficult to assess because few studies of nonresponders have been published [18]. Because treatment is associated with a variety of adverse effects, including flu-like syndrome, fatigue, headache, leukopenia, and psychiatric disturbances, [6, 17, 19], patients considering treatment need to be informed of both the probability of nonresponse and of seroconversion without treatment. Estimates of the latter number have not been readily available.

In this study, we estimated spontaneous HBeAg-negative seroconversion rates in HBV carriers of different ages in order to provide information useful in deciding whether to treat with interferon-\(\alpha\). Our study population was initially identified through screening and followed prospectively. Most other studies of the natural history of chronic HBV infection have used persons who initially presented with liver disease [6, 19]. Our study population was not selected for evidence of liver disease and would therefore be more representative of the population of HBV carriers overall. It is not, however, representative of those usually included in interferon-\(\alpha\) clinical trials where eligibility criteria required histologic or biochemical evidence of liver damage. The subset of our study population who had elevated ALT levels, however, represent that group of carriers who would be considered possible candidates for interferon-\(\alpha\) [6, 7].

We have shown that stable seroconversion is a relatively common event among HBeAg-positive carriers, occurring in

### Table 3. Spontaneous seroconversion versus interferon-\(\alpha\) therapy for chronic carriers of HBV with elevated alanine aminotransferase (ALT) levels.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Expected response in 15 months</th>
<th>Additional months needed before spontaneous seroconversion rate equals interferon-(\alpha) seroconversion rate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–19</td>
<td>15 (8–27)</td>
<td>18 (+2 to +52)</td>
</tr>
<tr>
<td>20–34</td>
<td>23 (15–34)</td>
<td>8 (–1 to +21)</td>
</tr>
<tr>
<td>35–50</td>
<td>17 (10–28)</td>
<td>17 (+3 to +40)</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval.
* Includes person-time from 1st elevated ALT level only.

### Table 2. Incidence rate of stable seroconversion of chronic HBV carriers per 10^5 person-months by age group and alanine aminotransferase (ALT) status.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>ALT (\leq 50) IU/mL at study entry</th>
<th>ALT &gt;50 IU/mL at study entry</th>
<th>ALT &gt;50 IU/mL at any time*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–19</td>
<td>166.0 (86.4–319.0)</td>
<td>993.2 (446.2–2210.9)</td>
<td>1067.3 (533.8–2134.3)</td>
</tr>
<tr>
<td>20–34</td>
<td>403.5 (234.3–694.9)</td>
<td>1773.7 (1007.4–3122.9)</td>
<td>1753.3 (1104.4–2783.3)</td>
</tr>
<tr>
<td>35–50</td>
<td>688.1 (399.6–1184.9)</td>
<td>870.4 (362.3–2091.1)</td>
<td>1257.2 (730.0–2164.9)</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval.
* Includes person-time from 1st elevated ALT level only.
50% of carriers by the age of 24. Although the incidence of stable seroconversion for children with normal ALT levels was low (~2%/year), in adults it occurred at ~5%–8%/year. Seroconversions occurred even more frequently in those with elevated ALT levels at study entry: ~11%/year for children and 10%–19%/year for adults. We estimated the potential efficacy of interferon-α therapy in the HBeAg-positive carrier population with elevated ALT levels by comparing expected rates of spontaneous seroconversion with the published response rates from clinical trials. The comparison between spontaneous and interferon-α–induced seroconversion rates can be expressed by two quantitative measures: the cumulative incidence of seroconversion expected during a fixed period and the number of months required to achieve a given probability of seroconversion (table 3).

The rather modest improvements of interferon-α therapy over spontaneous seroconversion must be interpreted with caution, particularly for children. Interferon-α therapy is most effective in HBV carriers with evidence of active viral replication and chronic active hepatitis. For such patients, the hastening of spontaneous seroconversion by even a few months through treatment may have substantial benefit [17]. For healthier carriers, however, treatment is not only unlikely to be effective, but its use in an early stage of the disease may also decrease response to later therapy because repeated courses have not been shown to be effective in nonresponders [20, 21].

What is unknown is whether interferon-α merely accelerates the natural process of seroconversion by a few months in those who would have seroconverted spontaneously or whether some of the patients who respond to therapy might never seroconvert otherwise. In our relatively healthy study population, 8% remained positive at age 50. No known indicators can predict which carriers will spontaneously seroconvert before the advent of serious liver disease and which are likely to remain HBeAg-positive throughout life. Until more becomes known about the effect of therapy on the natural history of chronic infection in both responders and nonresponders, a conservative approach to the use of interferon-α is appropriate. Of particular importance is the evaluation of the status of the patient’s underlying liver disease over a long period prior to the recommendation for therapy. Many patients have transient elevations of ALT level in the period immediately prior to spontaneous seroconversion [22, 23].

Our study population was composed of Asian-Americans. It has been reported that interferon-α therapy is less effective for Chinese patients and for patients who acquired infection early in life [8]. This association may, however, be artifactual since the three major randomized clinical trials of interferon-α involving Chinese patients have included HBV carriers with normal ALT levels. Most other randomized trials have included only patients with elevated ALT levels, a predictor of better response [6]. Lok et al. [24] found that 33% of Chinese subjects with elevated pretreatment ALT levels responded, a proportion equal to that of the other studies reviewed for the metaanalysis of Wong et al. [5]. The rates of spontaneous HBeAg-negative seroconversion observed in our study are similar to those reported for both Asian and non-Asian HBV carriers in other studies [3, 4, 22, 23, 25].

In our study, we have estimated the proportion of HBV carriers who will become HBeAg-negative before the age of 50. The clinical trials of interferon-α have estimated the proportion of HBeAg-positive carriers who will respond to treatment. What still remains unknown is whether the responders to interferon-α and those who will seroconvert spontaneously represent the same subpopulation of HBV carriers. Carefully designed long-term epidemiologic studies of nonresponders to interferon-α therapy would be of great value in answering this essential question.

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Appendix

Since the time variable of interest was age rather than time on study and ages of entry into the study varied, the survival analysis used left truncation and right censoring [10]. Time of infection was unknown for all subjects, but it was assumed that it occurred in infancy or early childhood, as is usual in populations in which HBV is endemic [26]. The age-specific incidence rates in table 2 were calculated using Poisson regression. In addition, we calculated the incidence rates nonparametrically using a kernel smoother for the hazard curve [11]. The results were similar to those produced by Poisson regression.

Two measures were used to compare the observed incidence rates of seroconversion to those expected from interferon-α therapy. The first was the cumulative incidence of seroconversion over a given time period. When the incidence rate (λ) of seroconversion is constant, the cumulative incidence (CI in formula) in a time interval (t) is estimated as

\[ CI = 1 - e^{-\lambda t} \]

The cumulative incidence can be interpreted as the average probability (p) for an individual of seroconverting within the interval t [27]. To estimate the additional time (T) required to achieve a probability of seroconversion equal to that of interferon-α therapy, we solve for t and subtract the median number of months (m) required for treatment and follow-up in the interferon-α clinical trials:

\[ T = -\frac{\ln(1 - p)}{\lambda} - m. \]

The validity of our method of estimation depends upon two assumptions. First, there should be no secular trend in the age-specific incidence rates during the time interval t. Second, competing risks must be small. Time intervals of interest were relatively short in this case, and it is unlikely that either a secular trend or competing risks significantly affected the results.
References