Reduction of chronic hepatitis B-related hepatocellular carcinoma with anti-viral therapy, including low risk patients

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SUMMARY

Background
Anti-viral therapy in chronic hepatitis B (CHB) is associated with a reduced risk of hepatocellular carcinoma (HCC) primary described in patients with cirrhosis.

Aim
To examine the effects of treatment on HCC incidence in CHB with and without cirrhosis, after adjustment for background risks.

Methods
A total of 2255 CHB patients from a US cohort (973 received anti-viral therapy) and 3653 patients from the community-based Taiwanese REVEAL-HBV study, none of whom received treatment. We used Cox proportional hazard models to calculate the risk of developing HCC after adjustment with the previously validated REACH-B risk score.

Results
We found 273 incident cases of HCC. After adjustment, therapy lowered the risk of HCC development in the US treated cohort when compared to the US untreated cohort (HR 0.31; 95% CI: 0.15–0.66; P = 0.002). HCC risk reduction was also confirmed when compared to the REVEAL cohort (HR 0.22; 95% CI: 0.12–0.40; P < 0.001). Each REACH-B point was associated with a 53% increased risk of HCC (HR 1.53; 95% CI 1.46–1.59; P < 0.001). We found a significant statistical reduction in HCC incidence with therapy regardless of gender, age, cirrhosis status, HBeAg serology, alanine aminotransferase level, REACH-B score or treatment medication. Therapy was beneficial to those with mildly- to moderately elevated HBV DNA levels (>2000 IU/mL) and of even greater benefit to those with levels >200 000 IU/mL.

Conclusion
After adjustment for background risk, anti-viral therapy was associated with a significant reduction in HCC incidence in both community and real-life clinical cohorts, including in those patients previously thought to be at low risk.
INTRODUCTION

Chronic hepatitis B (CHB) affects ~250 million people worldwide.1 While endemic to Asia and Africa, CHB is also prevalent in the USA in many immigrant groups, and was estimated to infect approximately 0.8 to 1.4 million persons in 2006,2 although this number is likely to be too low, due to an under-utilisation of diagnosis and treatment in immigrant populations.3

Treatment of CHB attempts to eliminate long-term complications, particularly the development of cirrhosis and hepatocellular carcinoma (HCC). Risk factors for CHB-related HCC include male gender, older age, presence of cirrhosis, hepatitis B e antigen (HBeAg) positivity, high serum hepatitis B virus (HBV) DNA levels and elevated serum alanine aminotransferase (ALT) levels.4–6 These risk factors are important parameters in the determination of eligibility for treatment following clinical guidelines,7–10 and have been incorporated into risk calculators for patient triage and treatment.

While studies examining the effect of anti-viral treatment have demonstrated a reduced risk of CHB-related HCC, this reduction has been noted most often in patients with cirrhosis.11–13 In addition, although some studies have matched probability of receiving treatment using a propensity-score matching method, adjustment for background risk such as cirrhosis has not been well addressed to date. A recent systematic review of both clinical trials and non-interventional studies by Lok et al. has shown anti-viral therapy to be helpful in lowering HCC risk in patients with cirrhosis. However, this beneficial effect has not yet been demonstrated consistently in patients with lower risk profiles, such as those without cirrhosis, females and patients with lower serum ALT.14 Another recent comprehensive review of anti-viral therapy and HCC risk from Europe reported similar findings.15

In 1991 and 1992, patients were recruited for the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer in Hepatitis B Virus (REVEAL-HBV) study in a Taiwanese-based prospective cohort of CHB patients who had never received anti-viral therapy.5 Using risk factors from the REVEAL study cohort, a clinically useful predictive score called the Risk Estimation for Hepatocellular Carcinoma in Chronic Hepatitis B (REACH-B), was developed and externally validated in a Hong Kong and South Korea cohort.16

In our study, we described the effect of anti-viral therapy in a large clinical US cohort, and also examined the REVEAL population-based cohort. The predominant study population consisted of patients without cirrhosis and background risk, and was adjusted using the REACH-B predictive score.

METHODS

US cohort study population

For the retrospective US cohort, we enrolled consecutive CHB patients meeting study inclusion criteria at two medical centres and two specialty community-based clinics in the San Francisco Bay Area from 1991 to 2014. Time of inclusion was defined as the date of the first clinic visit and length of follow-up was determined from this date. Treatment included peginterferon or any of the oral anti-HBV nucleoside/nucleotide analogue (NUCs). Initiation of therapy was at the discretion of the clinician, typically based upon American Association for the Study of Liver Disease (AASLD) or US Panel recommendations.7, 8

A thorough individual chart review of electronic medical records was conducted for each patient using a case report form. The online supporting information contains information regarding the REVEAL-HBV cohort and assessment criteria for the presence of cirrhosis and HCC.

This study was approved by the Institutional Review Board at Stanford University (Stanford, CA). Enrolment of the REVEAL cohort was performed under review by the Institutional Review Board of the College of Public Health at National Taiwan University (Taipei, Taiwan, Republic of China).

Patient selection

Eligibility criteria for the US cohort included adult CHB patients who were identified by either serology or International Classification of Disease 9 (ICD-9) codes (070.2x or 070.3x). The patients identified by ICD-9 codes were further confirmed to have CHB by serology.

Patients were excluded from the study if they received anti-viral treatment prior to presentation, had HCC at presentation or within the first year of follow-up (prevalent cancers), had less than 12 months of total follow-up or insufficient data, or were co-infected with human immunodeficiency virus, hepatitis C, or hepatitis D virus. In total, 2255 consecutive patients who met eligibility criteria were included in the primary analysis of this study.

Statistical analysis

In the analysis of baseline demographic and laboratory values in each group, categorical variables were described.
as proportions, while continuous variables were described as mean ± standard deviation. Baseline HBV DNA levels were log transformed, as this variable had a skewed distribution. Differences were analysed qualitatively and quantitatively between groups using the \( \chi^2 \) test or the Fisher exact test for categorical variables, and the Student’s \( t \)-test for continuous variables.

When comparing HCC risk in different groups, REACH-B scores of patients in all three groups (US treated, US untreated and REVEAL) were calculated according to a documented algorithm, using parameters before treatment including gender, age, HBeAg status and serum ALT. This score was validated to be accurate and reliable for the prediction of future HCC risk in Asian treatment-naive CHB patients. Distributions of REACH-B scores across the US treated and US untreated cohorts as well as the REVEAL cohort were compared using the ANOVA test. REACH-B scores for the US treated group and the combined untreated patients were compared using the Student’s \( t \)-test. Cumulative incidence of HCC in follow-up years was generated using the \textit{sts graph} syntax of the software STATA 11 (StataCorporation, College Station, TX, USA), with stratification according to treatment status [using the \texttt{strata()} option] and adjusted for REACH-B score [using the \texttt{adjustfor()} option]. Verification of proportionality assumption was carried out using Schoenfeld residuals followed by the chi-squared test, with no violation of this assumption observed. A Cox proportional hazard regression model was used to estimate the hazard ratio (HR) and 95% CI of developing HCC for various cohorts/treatment groups, and REACH-B scores were included in the model as a continuous variable for the adjustment. In subgroup analysis, the association between anti-viral treatment and HCC was examined in relation to cirrhosis status at entry, gender, age, HBeAg status, serum HBV DNA level, serum ALT level, REACH-B scores and treatment medication. The upper limit of normal (ULN) of ALT was defined as 30 U/L for men and 19 U/L for women, the same figures as those adopted by the AASLD. To compare treatment effects across subgroups, we used formal tests for interaction or heterogeneity to assess variations in treatment groups across subgroups. Results of subgroup analyses were summarised in a forest plot.

All statistical tests were two-sided, and significance was defined as a two-sided \( P < 0.05 \). Except for the cumulative incidence figure, all other statistical analyses were performed using SAS software (SAS Institute, Cary, NC, USA).

\section*{RESULTS}

\subsection*{Patient characteristics}

Table 1 presents demographic and baseline laboratory information for the entire cohort \((n = 5908)\). The overall cohort was further divided into three subgroups: US treated cohort, US untreated cohort and the REVEAL cohort.

Within the US cohort, the majority of patients were of Asian ethnicity and did not receive treatment (56.9%). Treated patients most frequently received the newer anti-viral agents, such as entecavir (46.9%), tenofovir (22.2%), adefovir (19%), lamivudine (10.5%), telbivudine (0.8%), emtricitabine (0.5%) and peginterferon (0.1%). The vast majority of treated patients (81%) achieved continuous virological suppression, defined as having undetectable HBV DNA for the duration of follow-up. The treated group was more likely to be male, with higher baseline ALT and HBV DNA levels.

The two cohorts that received no treatment (REVEAL and the US untreated group), differed in that the US untreated patients were more likely to have cirrhosis and higher baseline ALT and HBV DNA levels. These variables likely represent the clinic-based nature of the US cohort compared to the community-based REVEAL cohort.

Baseline risks were different in all REACH-B variables between those treated (US treated group) and those not receiving treatment (REVEAL and the US untreated group), indicating differences in background risk inherent between treated vs. untreated CHB patients.

\subsection*{Distributions of REACH-B score for various cohorts/groups}

Distribution of REACH-B scores across the US treated, US untreated and REVEAL cohort are shown in Figure 1 (Box plot included in online Supplementary Information as Figure S1). There were similar distributions between the US untreated group and the REVEAL cohort (all untreated), but these distributions were still different and statistically significant, due to the large sample size. However, the US treated group had higher REACH-B scores than either the US untreated group, the REVEAL cohort or the combined untreated patients (Figure 2; \( P < 0.001 \)) [Box plot included in online Supplementary Information as Figure S2]. The differences in REACH-B scores among cohorts provide strong justification for comprehensive adjustment of background risk in the evaluation of treatment efficacy.
HCC incidence

Kaplan–Meier curves adjusted for the REACH-B score show that treated patients had a statistically significant lower cumulative incidence of HCC than those not receiving treatment across the 10 years of follow-up (Figure 3; \( P < 0.001 \)). These rates are shown in Table 2. Overall, there were a total of 12 cases of HCC in the US treated cohort and 261 cases in the combined US
untreated and REVEAL cohorts. Overall median follow-up was 16.3 years. There were lower HCC rates in those receiving treatment than those who did not. To account for background risk differences between the cohorts, we utilised the REACH-B risk score adjustment. There was no difference in HCC incidence between the two untreated cohorts (USA and REVEAL) after adjustment for baseline risk (HR 1.41; 95% CI: 0.84–2.35; P = 0.19).

In the US treated cohort, anti-viral therapy lowered the risk of developing HCC by 69% compared to the US untreated group (HR 0.31; 95% CI: 0.15–0.66; P = 0.002), and also showed a 78% risk reduction when compared to the REVEAL cohort (HR 0.22; 95% CI: 0.12–0.40; P < 0.001). Treatment compared to the combined untreated cohort (US untreated and REVEAL patients) continued to show a statistically significant large HCC risk reduction of 77% (HR 0.23; 95% CI: 0.13–0.43; P < 0.001).

In addition, each point of the 17-point REACH-B score was associated with an approximate 50% increase in HCC incidence in all models (US treated vs. US untreated, US treated vs. REVEAL, REVEAL vs. US treated vs. REVEAL, HR 1.53; 95% CI: 1.47–1.59; P < 0.001).

**Treatment effect in subgroups**

In subgroup analysis, we examined the efficacy of treatment on HCC reduction with several major risk factors (Figure 4). Anti-viral therapy was beneficial regardless of gender, age (≤55 or >55 years), cirrhosis status, HBeAg serology, ALT levels (<ULN or >ULN), REACH-B scores (≤10, 11–13, or ≥14) or type of treatment medication, contrasting older oral anti-virals lamivudine or adefovir, with currently recommended first-line oral treatments entecavir or tenofovir. All tests for heterogeneity were not statistically significant,
indicating that the beneficial effect of anti-viral therapy was similarly robust in all tested subgroups (all $P > 0.05$). With regard to serum HBV DNA levels, patients with undetectable to low ($\leq 2000$ IU/mL) levels of viraemia did not demonstrate statistically significant benefit with treatment (HR 0.94; 95% CI: 0.12–7.23; $P = 0.95$). However, in subjects with levels greater than 2000 IU/mL and below 200 000 IU/mL, there was a clear-cut risk reduction with anti-viral therapy (HR 0.27; 95% CI: 0.10–0.74; $P = 0.01$). The benefit of anti-viral therapy was even more convincing in the treatment of patients with very high viral loads ($\geq 200$ 000 IU/mL; HR 0.20; 95% CI: 0.09–0.44; $P < 0.001$).
In patients with ALTs above the ULN, there was a large reduction in HCC incidence with treatment (HR 0.26; 95% CI: 0.13–0.52; \( P < 0.001 \)), but the HCC risk reduction remained statistically significant even in patients with ALTs below this strict ULN measurement (HR 0.16; 95% CI: 0.04–0.64; \( P = 0.01 \)).

We also found a better preventive effect with treatment for patients with lower REACH-B scores than those with higher REACH-B scores (HRs 0.18, 0.22 and 0.31, respectively, for patients with REACH-B scores \( \leq 10 \), 11–13 and \( \geq 14 \), although heterogeneity was not statistically significant (\( P = 0.75 \)).

**DISCUSSION**

Our study shows that anti-viral therapy was associated with a statistically significant HCC risk reduction in both clinical and community cohorts of patients with and without cirrhosis after adjustment for all major background risk factors using a previously validated HCC predictive risk score. A strong and statistically significant preventive effect of anti-viral therapy was also demonstrated in CHB patients without cirrhosis, a subgroup of patients not often studied in current literature. The benefit of treatment was observed irrespective of cirrhosis status, age, gender, HBeAg status, ALT level, REACH-B score or type of anti-viral medication. In addition, statistically significant risk reduction was observed even in treated subjects who had modestly elevated HBV DNA levels at baseline (\( \geq 2000 \) IU/mL).

Most studies with interferon anti-viral therapy have demonstrated a protective effect in HCC risk reduction, but this effect has been observed for the most part in patients with cirrhosis.\(^{18–20}\) Even with the new generation of NUCs, only a few long-term studies with

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**Figure 4 | Treatment effects on HCC development in various subgroups.**

| Stratifying variable | No. of HCC cases | P for interaction | P value | Hazard ratio (95% CI)* |
|----------------------|------------------|------------------|---------|-----------------------|
| Cirrhosis at entry   |                  |                  |         |                       |
| No                   | 229              | 0.41             | <0.001  |                       |
| Yes                  | 44               |                  | 0.03    |                       |
| Gender               |                  |                  |         |                       |
| Female               | 52               | 0.69             | 0.02    |                       |
| Male                 | 221              |                  | <0.001  |                       |
| Age                  |                  |                  |         |                       |
| \( \leq 55 \)        | 163              | 0.75             | 0.001   |                       |
| \( >55 \)            | 110              |                  | <0.001  |                       |
| HBeAg serostatus     |                  |                  |         |                       |
| Seronegative         | 159              | 0.55             | <0.001  |                       |
| Seropositive         | 114              |                  | 0.003   |                       |
| Serum ALT level, U/L |                  |                  |         |                       |
| \( \leq \text{ULN}^\dagger \) | 168 | 0.67 | 0.01 |                       |
| \( >\text{ULN}^\dagger \) | 105 |                  | <0.001  |                       |
| Serum HBV DNA level, |                  |                  |         |                       |
| IU/mL                |                  |                  |         |                       |
| 60 - <2,000          | 31               | 0.29             | 0.95    |                       |
| 2000 - <200,000      | 89               |                  | 0.01    |                       |
| \( \geq 200,000 \)   | 132              |                  | <0.001  |                       |
| REACH-B score        |                  |                  |         |                       |
| \( \leq 10 \)        | 114              | 0.75             | 0.02    |                       |
| 11–13                | 121              |                  | 0.001   |                       |
| \( \geq 14 \)        | 38               |                  | 0.02    |                       |
| Treatment medication |                  |                  |         |                       |
| LAM or ADV\(^\ddagger\) | 267 | 0.83 | <0.001 |                       |
| ETV or TDF\(^\ddagger\) | 266 |       | <0.001 |                       |

Abbreviations: ADV, adefovir; ALT, alanine aminotransferase; CI, confidence interval; ETV, entecavir; HBeAg, hepatitis B e antigen; LAM, lamivudine; TDF, tenofovir; ULN, upper limit of normal.

* Adjustment for REACH-B score as a continuous variable.

\^ ULN defined as 30 U/L for men and 19 U/L for women.

\ddagger Comparing with all untreated patients.
lamivudine have shown a benefit in those without cirrhosis. Current first-line agents, such as entecavir and tenofovir, are even more potent and produce low rates of resistance, yet so far studies have continued to show inconclusive data for those without cirrhosis.23, 24

A recent study based on a nationwide health insurance database in Taiwan showed the protective effect of anti-viral treatment on HCC in patients with and without cirrhosis.25 However, this study had vague definitions for CHB and cirrhosis, and lacked laboratory data crucial for the adjustment of background risk. Further complicating this analysis was that the untreated group received hepatoprotectants for at least 90 days, and had to demonstrate abnormal ALT levels in order to obtain medication reimbursement. These patients did not reflect typical CHB patients, and likely had higher risks for HCC. In addition, 60% of the treated patients received the older anti-viral lamivudine. The overwhelming majority of the patients in our study were treated with up-to-date NUCs, and our analysis showed definitive risk reduction in HCC when compared with both community and clinical untreated cohorts, including patients without cirrhosis, and after making important adjustments for predictors of HCC. Another recent study examining the effect of a first-line agent also observed a lower HCC incidence in treated patients than predicted. However, this study did not include a control group of untreated patients and was comprised solely of clinical trial patients, and thus was not a real-world patient cohort.26

As it is currently considered unethical to leave patients untreated given the known benefits of anti-viral therapy, there has been only one single randomised controlled trial comparing NUCs to placebo with regard to HCC incidence.21 Instead, contrasting treated patients with those in historically untreated control groups has been the most commonly adopted method of comparison. Based on the criteria for treatment recommendations by current professional societies, CHB patients who are selected for anti-viral treatment are generally expected to have higher risk profiles and an elevated risk of end-stage liver disease. Therefore, observational studies have attempted to adjust for bias due to confounding variables primarily by propensity-score matching, in which subjects are matched according to baseline factors that determine the probability of receiving therapy.24, 27–30 Unfortunately, the propensity-score matching approach does not necessarily guarantee comparable background risks between treated and untreated groups. In the present study, we used the externally validated REACH-B score in both Asian and North American populations,23, 31, 32 which directly places all cohorts in the same risk scale. While propensity-score matching attempts to estimate by indirectly accounting for the covariates that predict receiving treatment, REACH-B score adjustment directly and efficiently combines five known important predictors of HCC into a single variable. This scoring also preserves sample size, allowing further clinically pertinent subgroup analysis, including stratification by REACH-B scores.

A further advantage of using REACH-B scores was an improvement in prediction of HCC development. Those treated had higher REACH-B scores, and each point in this 17-point score was associated with an approximate 50% increase in risk (P < 0.001) across this community and clinical cohort.

In subgroup analysis, our results showed that patients with strong and independent predictors of increased HCC risk,4–6, 16 including male gender, older age, cirrhosis and high REACH-B scores, have all benefited from treatment. Importantly, we also found benefit from treatment in subjects who were historically considered to have low HCC risk factors, such as female gender, younger age, lack of cirrhosis, HBeAg negativity, normal to minimally elevated ALT levels, and low-to-middle REACH-B scores (<14). While technically considered to be at low risk, these patients still have a tangible risk of HCC and may also benefit from anti-viral treatment. Studies have shown that as many as 40% of immune tolerant patients with persistently normal ALT levels still have evidence of significant fibrosis or inflammation on biopsy.33, 34 It should also be noted that, while anti-viral therapy can reduce HCC risk, it does not eliminate the risk completely and treated patients should continue to undergo HCC surveillance.35 In addition, viral suppression induced by anti-viral therapy does not mean cure and long-term or lifelong therapy is often indicated, as HBVcure with HBsAg seroclearance is a very rare event in treated as well as untreated adult HBV patients, especially among Asian patients.36

The strengths of our study included large sample size, a diverse patient population from clinic and community centres in two countries, patients with and without cirrhosis, and background risk adjusted with a validated HCC risk score. This was a real-life cohort that was not restricted by a clinical trial environment. We also constructed precise definitions for cirrhosis and HCC that were obtained by individual medical record reviews.

Limitations of the study should be noted. European studies examining REACH-B have found the predictive score to be of limited efficacy in Caucasian patients.37, 38

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and thus our results may not apply to Caucasian CHB populations. However, Asians constitute a large majority of patients living with CHB worldwide, and in this study accounted for >95% of the US cohort and all of the REVEAL cohort. Also, the issue of heterogeneity may arise when combining US and Asian cohorts, as well as combining community and clinical cohorts. However, our analysis showed similar results of anti-viral effect on HCC incidence when the comparisons were limited only to US clinical cohorts (adjusted HR 0.31; CI 0.15–0.66; P = 0.002). Another limitation is that cirrhosis was defined by either radiographic imaging or histology, and the possibility exists that early cirrhosis not apparent on radiograph could have been missed.

In summary, this large cohort of 5908 CHB patients demonstrated that treatment with anti-viral therapy was statistically significant and independently associated with lower HCC risk after comprehensive adjustment for background risk. Treatment was associated with a large 77% HCC risk reduction in patients with only modestly elevated HBV DNA levels (>2000 IU/mL) regardless of differences in gender, age, cirrhosis status, HBeAg status, ALT level, REACH-B scores or treatment medication.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.

Figure S1. Distributions of REACH-B risk scores for three cohorts/groups (US untreated vs. US treated, P < 0.001; US untreated vs. REVEAL, P < 0.001; US treated REVEAL, P < 0.001).

Figure S2. Distributions of REACH-B risk scores for the US treated cohort compared to the combined US untreated + REVEAL cohort (P < 0.001).

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