의학박사 학위논문

면역 관용기 만성 B 형간염 환자의 간세포암 및 사망 발생의 위험성

High Risk of Hepatocellular Carcinoma and Death in Patients with Immune-Tolerant Phase Chronic Hepatitis B

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Abstract

Objective: High serum hepatitis B virus (HBV) DNA levels are associated with high risks of hepatocellular carcinoma (HCC) and cirrhosis in chronic hepatitis B (CHB) patients. Although the immune-tolerant (IT) phase is characterized by high circulating HBV DNA levels, it remains unknown whether antiviral treatment reduces risks of HCC and mortality.

Design: This historical cohort study included HBeAg-positive CHB patients with high HBV DNA levels (≥20,000 IU/mL) and no evidence of cirrhosis at a tertiary referral hospital in Korea from 2000 to 2013. The clinical outcomes of 413 untreated IT phase patients with normal alanine aminotransferase (ALT) levels (females, <19 IU/mL; males, <30 IU/mL) were compared with those of 1497 immune-active (IA) phase patients (ALT ≥80 IU/mL) treated with nucleos(t)ide analogs.

Results: The IT group was significantly younger than the IA group (mean age, 38 vs 40 years at baseline, p=0.04). The 10-year estimated cumulative incidences of HCC (12.7% vs 6.1%; p=0.001) and death/transplantation (9.7% vs 3.4%; p <0.001) were significantly higher in the IT group than the IA group. In multivariable analyses, the IT group showed a significantly higher risk of HCC (hazard ratio [HR], 2.54; 95% confidence interval [CI], 1.54-4.18) and death/transplantation (HR, 3.38; 95% CI, 1.85-6.16) than the IA group; which was consistently identified through inverse probability treatment weighting, propensity score-matched, and competing risks analyses.

Conclusions: Untreated IT phase CHB patients had higher risks of HCC and death/transplantation than treated IA phase patients. Unnecessary deaths could be prevented through earlier antiviral intervention in select IT phase patients.

Keywords: Antiviral treatment; HBeAg-positive; Hepatocellular carcinoma; Immune-active phase; Mortality.

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INTRODUCTION

High levels of serum hepatitis B virus (HBV) DNA (>10^4 copies/mL or >2,000 IU/mL) are associated with a high risk of hepatocellular carcinoma (HCC) and disease progression irrespective of serum alanine aminotransferase (ALT) levels, HBeAg, and cirrhosis in patients with chronic hepatitis B (CHB).[1, 2] Long-term suppression of HBV DNA with nucleos(t)ide analogs (NUC) reduces the risk of HCC and mortality in immune-active (IA) phase CHB patients.[3-7]

Chronic infection with HBV progresses through different phases. The first, which is the immune-tolerant (IT) phase, is characterized by high circulating HBV DNA and normal ALT levels. Antiviral treatment is generally not recommended for these patients by most practice guidelines because of the notion that the histologic activity is dormant and the risk of disease progression is low in the IT phase.[8-12]

However, recent studies have claimed that the histologic activity and HBV-specific immune responses do occur in the IT phase and are comparable to those occurring in the IA phase.[13-15] Moreover, a high level of chromosomal HBV DNA integration and clonal hepatocyte expansion was found in patients considered to be in the IT phase, indicating that hepatocarcinogenesis could be underway in these patients.[16] These findings suggest that therapeutic interventions to minimize further damage to the hepatocytes should be considered for IT phase patients. However, virtually no clinical evidence exists regarding whether long-term antiviral treatment of IT phase patients reduces the risk of HCC and mortality.[17] The practice guidelines thus encourage research to improve knowledge pertaining to the natural history and indications for treatment in HBeAg-positive IT phase patients.[12, 18, 19]

Therefore, we investigated the long-term risks of HCC and death/transplantation in IT phase patients. Due to the lack of treatment recommendations provided by the current

practice guidelines as well as reimbursement policies for IT phase patients in real-world practice settings, we compared the long-term outcomes of untreated IT phase patients with those of treated IA phase patients. We also determined the factors that identify patients at the high risk of clinical events.

METHODS

Study subjects

The study subjects were recruited from a historical cohort of 4965 HBeAg-positive, treatment-naïve, non-cirrhotic, adult CHB patients at Asan Medical Center, a 2700-bed academic tertiary referral hospital in Seoul, Korea, between January 2000 and December 2013 (Figure 1). All patients had serum HBV DNA levels of ≥20,000 IU/mL and were HBeAg positive at baseline. The cohort comprised patients who had no evidence of cirrhosis, had no history of cancer or organ transplantation, and were followed for at least 1 year. Cirrhosis was defined as the presence of any of the following criteria: coarse liver echotexture or nodular liver surface on ultrasonography, clinical features of portal hypertension (e.g., ascites, splenomegaly, and varices), or thrombocytopenia (<100,000/mm^3). Patients were excluded if they met any of the following criteria: positive serology for hepatitis C virus, human immunodeficiency virus, or other hepatotrophic viruses (n = 89) or prior antiviral treatment or current treatment with immunosuppressive agents (n = 341).

The following ALT levels were defined as normal according to the criteria of the American Association for the Study of Liver Diseases (AASLD): <19 U/L for females and <30 U/L for males.[12)] The phases of CHB infection were determined by ALT levels at baseline in accordance with previous suggestions.[12, 20)] The IT phase was defined as an ALT

level less than the upper limit of normal (ULN), the IA phase was defined as an ALT levels more than 2 × ULN, and the mildly active (MA) phase was defined as an ALT level above ULN but lower than 2 × ULN (1-2 × ULN).

Patients were observed for 1 year from baseline to be included in the study and were excluded in the following cases: transition from the IT to IA phase (n = 51), no initiation of oral NUC treatment in the IA phase (n = 1227), and transition from the MA to IA phase (n = 206). Consequently, a total of 3051 HBeAg-positive CHB patients comprised the study cohorts (Figure 1): IT group (n = 413), IA group treated with NUCs (n = 1497), and MA group (n = 1141). All patients in the IA group had ALT levels of ≥80 IU/mL, the reimbursement criteria, at initiation of NUC treatment; none received interferon therapy.

This study was approved by the Institutional Review Board of Asan Medical Center, and the requirement for informed consent from patients was waived.

Outcomes and follow-up evaluation

The outcomes of interest were the occurrence of HCC and death or liver transplantation. The index date was defined as the date when a patient underwent his or her first test for serum HBV DNA levels. The follow-up period for each patient was calculated from the index date to the date of diagnosis of HCC, death, transplantation, or the last follow-up (May 31, 2016). IT phase and MA phase patients who started antiviral treatment during follow-up were censored at 6 months after the time of the treatment initiation. All patients who started NUC were advised to continue the treatment even after HBeAg seroconversion until HBsAg seroclearance was achieved. Information about baseline characteristics, antiviral treatments, and clinical outcomes of the patients were obtained from electronic medical records. To verify the complete set of follow-up data, information on the vital status and primary diagnosis of HCC in all patients was validated by accessing the Korean National Health Insurance Service database, which covers >99% of the entire Korean population and

provides information on the vital status of the patients. This database contains a high HCC registration rate (96.5%) and highly accurate diagnoses and has been previously validated as a reliable resource for research.[21)]

The study patients had regular clinical assessments, including ALT, HBeAg, and HBV DNA levels every 3–6 months. Patients received regular surveillance for HCC using abdominal ultrasonography and serum alpha-fetoprotein levels at baseline and every 6 months. The diagnosis of HCC was based on histologic examination and/or typical features (nodule >1 cm with arterial hypervascularity and portal/delayed-phase washout) determined through dynamic computed tomography and/or magnetic resonance imaging.[18, 22)]

Serum assays

Serological markers, including HBsAg, anti-HBs, HBeAg, and anti-HBe, were detected using enzyme immunoassays (Abbott Laboratories, Chicago, IL, USA). Serum HBV DNA levels were measured using a hybrid capture assay (lower limit of detection, 20,000 IU/mL; Digene Diagnostics, Gaithersburg, MD, USA) before 2007, and then, using a real-time PCR assay (linear dynamic detection range, 15 IU/mL–10^9 IU/mL; Abbott Laboratories). HBV genotype was not determined because >98% of Korean patients with CHB have HBV genotype C2.[23)]

Statistical analysis

All patients who met the eligibility criteria were included in the analyses regardless of phase transition and treatment initiation during follow-up; they were analyzed in primary groups according to the principle of intent-to-treat analysis.

The baseline characteristics of the patients were compared using the chi-square test for categorical variables; *t*-test or one-way analysis of variance was used to compare continuous variables. Cumulative incidence curves for HCC and death or transplantation

were estimated using the Kaplan–Meier method. We employed a Cox proportional hazards regression model to determine the hazard ratios (HRs) for the incidence of HCC and death or transplantation. Multivariable regression models were employed with a backward variable elimination approach. The adjusted variables in the multivariable analysis were patient group, age, sex, platelet count, and serum levels of HBV DNA and albumin.

To reduce the effect of selection bias and potential confounders between the groups, differences in the baseline characteristics (except ALT levels) were adjusted through inverse probability treatment weighting (IPTW) and propensity score-matching analysis. The variables used to derive propensity scores were age; sex; serum levels of HBV DNA, albumin, and total bilirubin; platelet count; diabetes mellitus; and hypertension. In IPTW, each individual was weighted by the inverse probability of their current phase.[24] In the propensity score-matching analysis, we used nearest neighbor matching with a caliper size of 0.05 and matched IT and IA phases in both a 1:1 ratio and a 1:3 ratio. We considered the covariates as balanced because the absolute standardized difference between the two groups was <0.1. Competing risks analysis was conducted for the interpretation of the cumulative incidence of HCC, with adjustment for the probability of death and liver transplantation in the entire cohort.

All reported p-values are two-sided, and p-values of <0.05 were considered significant. SAS (version 9.1, SAS, Cary, NC) and R (version 3.0, http://cran.r-project.org/) software were used for statistical analyses. R packages of CBPS and Matchit were used for the IPTW and matching analyses, respectively.

RESULTS

Characteristics of the study population

The primary study population comprised 413 IT phase patients and 1497 IA phase patients treated with NUCs (Figure 1). Baseline characteristics of the patients are shown in Table 1. The two groups had comparable proportions of males (66.8% vs 65.0%, p=0.49) and HBV DNA levels (median, 8.0 vs 7.7 $\log_{10}$ IU/mL, p=0.20). The IT group was significantly younger (mean age, 38 vs 40 years, p=0.04) and had higher albumin levels (median, 4.0 vs 3.9 g/dL, p<0.001) and platelet counts (median, 204 vs 181 × 1000/mm^3, p<0.001) compared with the IA group (Table 1).

All IA group patients received NUC treatment and were advised to continue the treatment even after the achievement of HBeAg seroconversion. NUCs initially administered to the IA group patients comprised lamivudine (n=872; 58.2%), entecavir (n=486, 32.5%), tenofovir (n=70, 4.7%), adefovir (n=46; 3.1%), clevudine (n=19, 1.3%), and telbivudine (n=4; 0.3%).

Clinical events in the IT vs. IA groups

Among 1910 patients in the IT and IA groups, 78 (4.1%) developed HCC and 52 (2.7%) died or received liver transplantation during the 6.3-years of median follow-up period. The annual HCC incidence rate was significantly higher in the IT group compared with the IA group (1.05% vs 0.51%; p=0.001), and the annual rate of death/transplantation was also significantly higher in the IT group than the IA group (0.76% vs 0.32%; p=0.001; Table 2).

The cumulative incidence of HCC and death/transplantation was significantly higher in the IT group compared with the IA group (Figure 2). The estimated cumulative incidence of HCC in the IT vs IA groups was 4.2% vs 1.6% at 5 years and 12.7% vs 6.1% at 10 years (p=0.001). The estimated cumulative incidence of death/transplantation in the IT vs IA groups was 1.9% vs 0.8% at 5 years and 9.7% vs 3.4% at 10 years (p<0.001). The estimated cumulative incidence of any clinical events (HCC, death, or transplantation) in the IT vs IA groups was 5.3% vs 2.2% at 5 years and 16.9% vs 7.7% at 10 years (p<0.001).

Multivariable analyses showed that the IT group was associated with a significantly higher risk of HCC (hazard ratio [HR], 2.54; 95% confidence interval [CI], 1.54–4.18; p<0.001) and death/transplantation (HR 3.38; 95% CI, 1.85–6.16; p<0.001) compared with the IA group (Table 3).

Inverse probability treatment weighting (IPTW) analysis

To reduce the effect of selection bias and potential confounders between the groups, IPTW was employed using propensity scores of patients at baseline. In IPTW, each individual was weighted by the inverse probability of their basal phase, and the baseline characteristics (except for ALT levels) of the IT and IA groups were well-balanced (Table 4). In this analysis, the IT group had a significantly higher risk of both HCC (HR, 2.69; 95% CI, 1.63–4.45; p < 0.001) and death/transplantation (HR 3.34; 95% CI, 1.83–6.11; p<0.001) than the IA group (Table 5).

Propensity score-matched analysis

After propensity score matching of the patients in the IT and IA groups, there was no significant between-group difference in baseline characteristics, except for ALT levels (Table 4). In the propensity score matched cohort on 1:1 ratio, the IT group showed a significantly higher risk of HCC (HR 2.43; 95% CI, 1.23–4.78; p=0.01) and death/transplantation (HR 2.80; 95% CI, 1.26–6.24; p=0.01) than the IA group (Table 5 and Figure 3). The risk of HCC and death/transplantation of IT group was consistently higher on 1:3 ratio than those of the IA group (HR 2.89; 95% CI, 1.67–4.99; p<0.001, HR 3.56; 95% CI, 1.85–6.86; p<0.001).

Competing risk analysis

HCC, death, and transplantation were the three competing outcomes of this study. It is

conceivable that the frequent occurrence of death or transplantation may have lowered the incidence of HCC. Thus, the risk of HCC was adjusted for the risk of death and transplantation using competing risks analysis. After the adjustment, the IT group had a significantly higher risk of HCC than the IA group (HR, 2.09; 95% CI, 1.08–4.05; p=0.03; Table 5).

Risk of clinical outcomes in the MA phase group

The MA phase was defined as elevated ALT levels more than ULN but less than 2 × ULN (1-2 × ULN) in HBeAg-positive non-cirrhotic patients with HBV DNA levels of ≥20,000 IU/mL (Figure 1 and Table 6).

Compared with the IA group, the MA group showed a significantly higher cumulative incidence of HCC (HR, 3.23; 95% CI, 2.28–4.57; p<0.001) and death/transplantation (HR, 3.21; 95% CI, 3.07–4.95; p<0.001; Table 2 and Figure 4).

Factors predictive of clinical events

Among the 1554 patients in the IT and MA groups, clinical events occurred in 130 patients (HCC, 82.3%; death/transplantation, 17.7%) during a median follow-up period of 4.3 years (IQR, 2.2–7.5 years), when only the initial events were considered. The estimated 5- and 10-year cumulative incidence of any clinical events in the IT and MA groups was 6.0% and 17.8%, respectively, which was significantly higher than 2.2% and 7.7%, respectively, in the IA group (p<0.001; Figure 5).

Older age (year; HR, 1.07), male sex (HR, 2.30), lower HBV DNA levels (log_{10} IU/mL; HR, 0.62), and lower platelet counts (×1,000/mm^3; HR, 0.99) were independently associated with a significantly higher risk of clinical events in the multivariable analysis (Table 7 and Figure 6, A-C).

Phase transition in the IT and MA groups

Of the 413 patients in the IT group, 108 (26.2%) started NUC at a median follow-up period of 4.7 years (IQR, 2.5–7.9 years) due to transition to the IA phase. The cumulative probabilities for starting NUC treatment at 5 and 10 years from baseline were 18.4% and 44.5%, respectively, according to Kaplan–Meier analysis (Figure 7).

Of the 1141 patients in the MA group, 371 (32.5%) started NUC treatment at a median follow-up period of 3.6 years (IQR, 2.0–6.5 years) due to transition to the IA phase. The cumulative probabilities for starting NUC treatment at 5 and 10 years from baseline were 28.3% and 53.1%, respectively, according to Kaplan–Meier analysis, and were significantly higher than the probabilities in the IT group (p=0.002; Figure 7).

Characteristics of the patients who developed HCC in the IT and MA groups

A total of 107 patients in the IT and MA groups developed HCC during median 5 years of follow-up from baseline. Their characteristics at baseline and at diagnosis of HCC are shown in Table 8. At diagnosis of HCC, the patients were significantly older; had significantly decreased levels HBV DNA, albumin; significantly decreased platelet counts; and significantly increased levels of ALT, compared with baseline. However, ALT levels remained < x2 ULN in 79.8% of the patients. From the non-tumor liver tissues of the 45 patients who received surgical resection (n=42) or transplantation (n=3), the METAVIR scores were assessed, and 31 (68.9%) had F3 or F4 fibrosis (Table 9).

DISCUSSION

We compared the long-term risks of HCC and death/transplantation in the untreated IT phase patients with those in the IA phase patients treated with NUC. This observational study

showed that the untreated IT group had a significantly higher risk of HCC and death/transplantation than the treated IA group. These results were consistently observed in the unadjusted, multivariable-adjusted, IPTW, propensity score-matched, and competing risk analyses.

All IT phase patients included in this study were non-cirrhotic adults with serum HBV DNA levels of ≥20,000 IU/mL, positive HBeAg, and normal ALT levels according to AASLD criteria, and maintained persistently normal ALT levels for at least 1 year from baseline. The IT group was significantly younger and had higher albumin levels and platelet counts compared with the IA group at baseline, all of which favor the lower risk of HCC in the IT group. Nevertheless, the estimated 10-year risk of clinical events (HCC, death, or transplantation) in the untreated IT group was significantly higher (17.8%) than that in the treated IA group (7.7%). Majority of the initial clinical events were HCC (82.3%) among the patients in the IT and MA groups.

Natural history studies have found a strong association between serum HBV DNA levels and the development of HCC and cirrhosis irrespective of serum ALT levels in adults.[1, 2] This raises the issue of whether our patients in the IT phase would benefit from antiviral therapy. Yet, there have been no studies demonstrating that antiviral therapy is beneficial for reducing rates of HCC and overall mortality in IT phase patients. The few short-term intervention studies in adults with IT phase CHB evaluated only surrogate endpoints such as virologic and serologic responses.[25] Given the lack of evidence that antiviral therapy is beneficial for reducing clinical events in IT phase patients, it has been considered that the potential harms of long-term therapy, including cost, drug adverse effects, and development of resistance, outweigh its benefits.[10, 12, 19, 26, 27] In this regard, our data may provide novel information suggesting earlier treatment initiation in IT and MA phase patients.

With the availability of potent and safe oral NUCs, such as tenofovir and entecavir, the suppression of HBV DNA replication is achievable in most patients without the risk of

drug resistance.[28, 29] The treatment of immunotolerant patients with an even less potent, low genetic barrier drug (lamivudine) was suggested to be highly cost effective in preventing HCC and cirrhosis.[30] Nonetheless, HBsAg seroclearance is very rarely achievable,[31, 32] and the long-term, almost indefinite NUC therapy is required in most patients. The treatment should be prolonged or indefinite, particularly in young IT phase patients, because the probability of HBeAg seroconversion and HBsAg seroclearance in these patients is extremely low. Furthermore, not all patients will develop cirrhosis and/or HCC in the long term. Therefore, factors to identify individuals at the high risk of disease progression and who would benefit from treatment should be discovered to ensure cost-effectiveness.[12, 18, 19]

The practice guidelines recommend that moderate-to-severe necroinflammation or fibrosis (determined through liver biopsy in adults with an IT profile) be used as an indication to start antiviral therapy.[12] However, this recommendation is supported by weak evidence and is not realistic given the risks and costs of repeat liver biopsy. Thus, serum ALT levels have been used as a surrogate marker of liver cell damage to evaluate the severity of hepatitis activity.[17, 33] However, as shown in this study, even among patients with persistently normal ALT levels, 28%–60% may have significant hepatic necroinflammation and/or fibrosis.[13-15, 34, 35] These results suggest that ALT levels should be considered as an imperfect surrogate marker for liver disease activity.

We identified age, sex, and platelet count as independent predictors of composite clinical events in IT and MA phase patients. In addition, lower HBV DNA levels were associated with significantly higher risks of HCC and death during follow-up. Notably, all our patients had HBV DNA levels of >20,000 IU/mL. The REVEAL-HBV studies on the natural history of HBV infection showed a direct correlation between serum HBV titers and the risk of HCC. The risk of HCC was highest with HBV titers above 10^5 copies/mL (approximately 20,000 IU/mL). However, virus titers above 10^6 copies/mL were not quantified, and most of the patients were HBeAg-negative (85%), with a median age of 45

years. The progression of these HBeAg-negative patients would not be identical to that of young HBeAg-positive IT phase patients with very high HBV DNA levels.[17, 33] Therefore, results from the REVEAL study could not be extrapolated to patients in the IT phase. In fact, the very high virus titers (>8 $\log_{10}$ IU/mL) often seen in immunotolerant young patients are not generally considered an HCC risk factor.[10, 12, 36] Instead, lower viral titers above 20,000 IU/mL may reflect a higher HCC risk because they also reflect cumulative immune damage to the infected liver.[16, 36, 37] In fact, subsequent studies from the REVEAL cohort also showed that compared with those with persistent HBV DNA levels $>10^7$ copies/mL, patients with HBV DNA levels at 10^5–10^7 copies/mL (approximately 4–6 $\log_{10}$ IU/mL) had a higher risk of HCC, [38, 39] which was consistent with our results.

Clonal hepatocyte repopulation is a major risk factor for HCC and may be important in the etiology of HBV-associated HCC even before the appearance of raised ALT levels and fibrotic or cirrhotic lesions.[37] Clonal hepatocyte repopulation is a genetic narrowing of the hepatocyte population that occurs in response to either endogenous or exogenous injury, with sensitive hepatocytes dying and being replaced by the clonal proliferation of hepatocytes that may evolve to form HCC.[40, 41] Immune killing of infected hepatocytes is the strongest known pressure on the infected hepatocyte population and may lead to the emergence and clonal proliferation of HBV-resistant hepatocytes that are able to avoid immune killing.[40, 42] Most analyses of long-term HBV carriers suggest that ≥50% of hepatocytes no longer support HBV infection or support highly reduced levels of replication.[40, 43] Therefore, there would be a possibility that the reduction in HBV titers, but above 20,000 IU/mL, in HBeAg-positive CHB patients could be an indication to initiate antiviral therapy even with persistently normal ALT levels. However, this hypothesis should be confirmed in future randomized controlled trials.

This study has several limitations. First, the IA phase patients were used as the treatment comparison group for IT phase patients. Because of the lack of treatment

recommendations in the practice guidelines and reimbursement policies for IT phase patients, very few patients received treatment in IT phase. We also considered that the inclusion of the very few treated IT phase patients may result in serious selection bias. Therefore, this multiple cohort study was considered the only feasible option. Although treated IA phase patients may not represent the treated IT group, their risk of clinical events of the IA group is unlikely to be lower than that of the IT phase patients if left untreated. Second, as an observational study, the findings were potentially subject to selection bias and confounding. Multiple strategies were used to rigorously adjust for differences in baseline susceptibility to the tested outcomes, including multivariable adjustment, propensity score matching, IPTW, and competing risk analyses. Nevertheless, unmeasured confounds could not be completely accounted for; further randomized trials are needed to ensure that our findings replicate. Despite this limitation, given the relatively low incidence of clinical events, the present observational study approach would arguably remain the best for comparing the effectiveness of oral antiviral agents.[44-47] Third, this real-world study used clinical and radiological criteria for diagnosing cirrhosis. Thus, there is a possibility that some patients with advanced fibrosis were included. Fourth, being a single-center study, the results of the current study may not be generalizable. Majority of the patients in the current study were infected with genotype C HBV that was acquired through vertical transmission,[23] both of which were previously proposed to be associated with a higher risk of HCC.[48, 49] Finally, new emerging biomarkers for HBV infection, such as quantitative HBsAg, Hepatitis B core-related antigen, and HBV RNA levels, that may help stratify the patients for the risk of clinical outcomes,[50, 51] could not be incorporated in the analyses because most of our study patients were recruited before the availability of those markers.

CONCLUSION

In conclusion, the present cohort study showed that the untreated patients in the IT phase had significantly higher risks of HCC and death/transplantation than the IA phase patients treated with NUCs. Our results suggest that many unnecessary deaths could be prevented by earlier antiviral intervention in the IT phases before the appearance of clinically active liver disease. Further studies taking new emerging biomarkers for HBV infection into account would be warranted to further stratify the patients for the risk of clinical events. Randomized controlled trials evaluating the long-term clinical outcomes of the IT phase patients with or without antiviral treatment may be worthwhile.

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국문요약

배경: 만성 B 형간염 환자에서 높은 혈청 HBV DNA 는 간세포암 및 간경화의 위험성이 높은 것과 연관되어 있다. 면역 관용기 (immune-tolerant phase) 만성 B 형간염 환자는 높은 혈청 HBV DNA 가 특징적이지만, 이 환자들에게 항바이러스 치료를 하는 것이 간세포암 혹은 사망의 위험을 줄이는지는 잘 알려져 있지 않다.

방법: 2000년부터 2013년까지 한국의 3차 병원에서 간경화의 증거가 없고, 높은 HBV DNA(≥20,000 IU/mL) 혈청 역가를 가지는 HBeAg 양성 만성 B형간염 환자를 대상으로 한 후향적 코호트 연구이다. ALT 수치가 정상이며 치료 받지 않은 면역 관용기 환자 413명과 항바이러스제 치료를 받은 1497명의 면역 제거기 (immune-active phase) 환자를 대상으로 임상 예후를 비교하였다.

결과: 면역 관용기 환자는 면역 제거기 환자보다 유의하게 평균 연령이 낮았다 (평균 나이 38세 vs 40세, p=0.04). 10년 간세포암 누적 발생률 (12.7% vs 6.1%; p=0.001) 및 10년 사망/이식 누적 발생률 (9.7% vs 3.4%; p<0.001)은 면역 관용기 환자에서 면역 제거기 환자보다 유의하게 높았다. 다 변량 분석에서 면역 관용기 환자에서는 면역 제거기 환자 보다 유의하게 간세포암 (hazard ratio [HR], 2.54; 95% confidence interval [95% CI], 1.54-4.18) 및 사망/이식 (HR, 3.38; 95% CI, 1.85-6.16)의 위험도가 높았다. Inverse probability treatment weighting 분석, propensity score matching 분석, competing risks analysis에서 동일한 결과가 확인되었다.

결론: 치료 받지 않은 면역 관용기 환자에서는 치료 받은 면역제거기 환자보다 간세포암 및 사망/이식의 발생이 높았다. 선별된 면역 관용기 환자에게 초기에 항바이러스제 치료를 통해 불필요한 사망을 예방할 수 있을 것으로 생각된다.

중심단어: 항바이러스 치료; HBeAg 양성; 간세포암; 면역 제거기; 사망

Table 1. Baseline characteristics of the study patients

Characteristic	IT Phase Group	IA Phase Group	p Value
No.	413	1497	
Age, mean ± SD, years	38 ± 11	40 ± 11	0.04
Male sex	276 (66.8%)	973 (65.0%)	0.49
HBV DNA, median (IQR), $\log_{10}$ IU/mL	8.0 (7.0–8.4)	7.7 (6.9–8.3)	0.20
4.00–6.99	108 (26.2%)	428 (28.6%)	
7.00–7.99	105 (25.4%)	516 (34.5%)	
≥8.00	200 (48.4%)	553 (36.9%)	
ALT, median (IQR), IU/mL	19 (16–25)	156 (95–308)	<0.001
AST, median (IQR), IU/mL	25 (21–31)	113 (69–216)	<0.001
Albumin, median (IQR), g/dL	4.0 (3.8–4.3)	3.9 (3.7-4.1)	<0.001
Total bilirubin, median (IQR), mg/dL	0.9 (0.7–1.1)	1.0 (0.8–1.3)	<0.001
Platelets, median (IQR), ×1000/mm^3	204 (167–242)	181 (149–214)	<0.001
Diabetes mellitus	7 (1.7%)	51 (3.4%)	0.07
Hypertension	19 (4.6%)	56 (3.7%)	0.43
Duration of follow-up period*, median (IQR), years	4.9 (2.4–8.6)	6.7 (3.7–10.3)	<0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; IA, immune-active; IQR, interquartile range; IT, immune-tolerant; SD, standard deviation.

*IT phase patients who started antiviral treatment during follow-up were censored at 6 months after the time of the treatment initiation.

Table 2. Incidence rates of hepatocellular carcinoma and death or transplantation in the entire cohorts.

Hepatocellular carcinoma					
Groups	**Patient -years**	**No. of events**	**No./100 patient-years (95% CI)**	**HR (95% CI)**	**p Value**
Immune-active phase, treated	10,633	54	0.51 (0.39–0.66)	Reference	–
Immune-tolerant phase, untreated	2,275	24	1.05 (0.71–1.57)	2.23 (1.38–3.61)	0.001
Mildly active phase, untreated	5,783	83	1.44 (1.16–1.78)	3.23 (2.28–4.57)	<0.001
Death or transplantation					
Groups	**Patient -years**	**No. of events**	**No./100 patient-years (95% CI)**	**HR (95% CI)**	**p Value**
Immune-active phase, treated	10,786	34	0.32 (0.23–0.44)	Reference	–
Immune-tolerant phase, untreated	2,357	18	0.76 (0.48–1.20)	2.73 (1.54–4.84)	0.001
Mildly active phase, untreated	6,037	52	0.86 (0.66–1.13)	3.21 (3.07–4.95)	<0.001

CI, confidence interval; HR, hazard ratio.
Cox proportional hazards models was used to represent the hazards ratios and p values.

Table 3. Factors predictive of hepatocellular carcinoma and death or transplantation in immune-tolerant and immune-active phase patients

Hepatocellular carcinoma*				
Variables	**Univariate analysis**		**Multivariable analysis**	
	HR (95% CI)	**p Value**	**HR (95% CI)**	**p Value**
Immune-tolerant phase group‡	2.23 (1.38–3.61)	0.001	2.54 (1.54–4.18)	<0.001
Age, years	1.08 (1.06–1.11)	<0.001	1.08 (1.06–1.11)	<0.001
Male sex	2.01 (1.14–3.53)	0.02	2.14 (1.21–3.79)	0.009
HBV DNA, $\log_{10}$ IU/mL	0.55 (0.45–0.68)	<0.001	0.63 (0.52–0.77)	<0.001
Albumin, g/dL	0.89 (0.50–1.57)	0.68	1.37 (0.75–2.51)	0.31
Platelets, ×1000/mm^3	0.98 (0.98–0.99)	<0.001	0.99 (0.98–0.99)	<0.001
Death or transplantation†				
Variables	**Univariate analysis**		**Multivariable analysis**	
	HR (95% CI)	**p Value**	**HR (95% CI)**	**p Value**
Immune-tolerant phase group‡	2.73 (1.54–4.84)	0.001	3.38 (1.85–6.16)	<0.001
Age, years	1.10 (1.08–1.13)	<0.001	1.10 (1.06–1.13)	<0.001
Male sex	1.54 (0.81–2.94)	0.19	1.83 (0.95–3.54)	0.07
HBV DNA, $\log_{10}$ IU/mL	0.63 (0.49–0.81)	<0.001	0.73 (0.56–0.94)	0.02
Albumin, g/dL	0.27 (0.15–0.48)	<0.001	0.34 (0.18–0.64)	0.001
Platelets, ×1000/mm^3	0.99 (0.98–0.99)	<0.001	0.99 (0.99–0.998)	0.01

CI, confidence interval; HBV, hepatitis B virus; HR, hazard ratio.
A Cox proportional hazards model with a backward elimination approach was used for multivariable analysis.
*Total number of patients, 1910; number of events, 78.
†Total number of patients, 1910; number of events, 52.
‡Treated immune-active phase group as a reference.

Table 4. Baseline characteristics of the propensity score-adjusted immune-tolerant phase and immune-active phase groups

	Before weighting			Inverse probability treatment weighting*			Propensity score-matched cohort†		
Characteristic	**Immune-tolerant phase group**	**Immune-active phase group**	**Standardized difference**	**Immune-tolerant phase group**	**Immune-active phase group**	**Standardized difference**	**Immune-tolerant phase group**	**Immune-active phase group**	**Standardized difference**
No.	413	1497	-	413	1497	-	397	397	-
Age, mean ± SD, years	38 ± 11	40 ± 11	0.12	38 ± 12	39 ± 11	0.01	39 ± 12	39 ± 11	-0.01
Male sex	276 (66.8%)	973 (65.0%)	-0.04	276 (66.8%)	1008 (67.4%)	0.01	267 (67.3%)	259 (65.2%)	-0.04
HBV DNA, median (IQR), $\log_{10}$ IU/mL	8.0 (7.0–8.4)	7.7 (6.9–8.3)	-0.07	8.0 (6.6–9.4)	7.7 (6.2–9.2)	-0.04	8.0 (7.0–8.4)	7.8 (6.9–8.4)	0.04
Albumin, median (IQR), IU/mL	4.0 (3.8–4.3)	3.9 (3.7-4.1)	-0.29	4.0 (3.5–4.5)	4.0 (3.5–4.5)	-0.02	4.0 (3.8–4.2)	4.0 (3.8–4.3)	0.04
Total bilirubin, median (IQR), mg/dL	0.9 (0.7–1.1)	1.0 (0.8–1.3)	0.43	0.9 (0.5–1.3)	0.9 (0.5–1.3)	0.09	0.9 (0.7–1.2)	1.0 (0.8–1.1)	0.02
Platelets, median (IQR), $\times 1000/mm^3$	204 (167–242)	181 (149–214)	-0.42	204 (129–279)	200 (125–275)	-0.09	202 (165–239)	200 (174–234)	0.01
Diabetes mellitus	7 (1.7%)	51 (3.4%)	-0.08	7 (1.7%)	28 (1.9%)	-0.05	7 (1.8%)	8 (2.0%)	0.02
Hypertension	19 (4.6%)	56 (3.7%)	-0.04	19 (4.6%)	63 (4.2%)	0.01	16 (4.0%)	19 (4.8%)	0.04

*Data were evaluated by chi-square test for categorical variables and by independent t-test or Mann–Whitney U test for continuous variables.
†Data were evaluated by McNemar test for categorical variables and by paired t-test for continuous variables.
HBV, hepatitis B virus; IQR, interquartile range; SD, standard deviation

Table 5. Comparison of outcomes in untreated immune-tolerant phase vs. treated immune-active phase by unadjusted and adjusted analyses

Hepatocellular carcinoma		
Model	**HR (95% CI)**	**p Value**
Unadjusted	2.23 (1.38–3.61)	0.001
Multivariable Cox regression	2.54 (1.54–4.18)	<0.001
IPTW analysis	2.69 (1.63–4.45)	<0.001
PS-matched analysis	2.43 (1.23–4.78)	0.01
Competing risk analysis	2.09 (1.08–4.05)	0.03
Death or transplantation		
Model	**HR (95% CI)**	**p Value**
Unadjusted	2.73 (1.54–4.84)	0.001
Multivariable Cox regression	3.38 (1.85–6.16)	<0.001
IPTW analysis	3.34 (1.83–6.11)	<0.001
PS-matched analysis	2.80 (1.26–6.24)	0.01

CI, confidence interval; HR, hazard ratio; IPTW, inverse probability treatment weighting; PS, propensity score
The risks of immune-tolerant phase group were compared to those of treated immune-active phase group as a reference.

Table 6. Baseline characteristics of the immune-tolerant vs. immune-active vs. mildly active phase patients

Characteristic	IT phase group	IA phase group	MA phase group	p Value
No.	413	1497	1141	-
Age, mean ± SD, years	38 ± 11	40 ± 11	40 ± 11	0.007
Male sex	276 (66.8%)	973 (65.0%)	676 (59.2%)	0.002
HBV DNA, median (IQR), log_{10} IU/mL	8.0 (7.0–8.4)	7.7 (6.9–8.3)	7.9 (6.7–8.5)	0.45
4.00–6.99	108 (26.2%)	428 (28.6%)	359 (31.5%)	
7.00–7.99	105 (25.4%)	516 (34.5%)	287 (25.2%)	
≥ 8.00	200 (48.4%)	553 (36.9%)	495 (43.4%)	
ALT, median (IQR), IU/mL	19 (16–25)	156 (95–308)	35 (29–45)	<0.001
AST, median (IQR), IU/mL	25 (21–31)	113 (69–216)	32 (27–40)	<0.001
Albumin, median (IQR), IU/mL	4.0 (3.8–4.3)	3.9 (3.7-4.1)	4.0 (3.8–4.2)	<0.001
Total bilirubin, median (IQR), mg/dL	0.9 (0.7–1.1)	1.0 (0.8–1.3)	0.9 (0.7–1.1)	<0.001
Platelets, median (IQR), ×1,000/mm^3	204 (167–242)	181 (149–214)	200 (163–235)	<0.001
Diabetes mellitus	7 (1.7%)	51 (3.4%)	30 (2.6%)	0.15
Hypertension	19 (4.6%)	56 (3.7%)	52 (4.6%)	0.52
Duration of follow-up*, median (IQR), years	4.9 (2.4–8.6)	6.7 (3.7–10.3)	4.4 (2.3–7.8)	<0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; IA, immune-active; IQR, interquartile range; IT, immune-tolerant; MA, mildly-active; SD, standard deviation

*IT and MA phase patients who started antiviral treatment during follow-up were censored at 6 months after the time of the treatment initiation.

Table 7. Factors predictive of composite clinical endpoints (hepatocellular carcinoma, death, or transplantation) in immune-tolerant and mildly active phase patients

Variables	Univariate analysis		Multivariable analysis	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Age, years	1.08 (1.07–1.10)	<0.001	1.07 (1.05–1.09)	<0.001
Male sex	2.53 (1.66–3.85)	<0.001	2.30 (1.50–3.52)	<0.001
HBV DNA, $\log_{10}$ IU/mL	0.51 (0.44–0.58)	<0.001	0.62 (0.53–0.73)	<0.001
Albumin, g/dL	0.44 (0.29–0.66)	<0.001	0.64 (0.39–1.05)	0.08
Platelets, ×1000/mm^3	0.98 (0.98–0.98)	<0.001	0.99 (0.99–0.99)	<0.001

CI, confidence interval; HBV, hepatitis B virus; HR, hazard ratio.
Total number of patients, 1554; number of events, 130.
A Cox proportional hazards model with a backward elimination approach was used for multivariable analysis.

Table 8. Characteristics of the IT and MA group patients who developed HCC during follow-up.

Characteristic	At baseline	At HCC diagnosis*	p Value
No.	107	107	
Age, mean ± SD, years	49 ± 8	54 ± 8	<0.001
Male sex	86 (80.4%)	86 (80.4%)	–
HBV DNA, median (IQR), log_{10} IU/mL	6.5 (6.0–7.0)	5.0 (2.2–6.4)	<0.001
ALT, median (IQR), IU/mL	35 (28–48)	39 (28–52)	0.004
<1 x ULN	24 (22.4%)	19 (18.3%)	
1–2 × ULN	83 (77.6%)	64 (61.5%)	
>2 × ULN	0	21 (20.2%)	
AST, median (IQR), IU/mL	40 (34–50)	40 (33–56)	0.005
Albumin, median (IQR), g/dL	3.9 (3.7-4.2)	3.7 (3.3-4.0)	<0.001
Total bilirubin, median (IQR), mg/dL	1.1 (0.8–1.3)	1.0 (0.7–1.4)	0.10
Platelets, median (IQR), ×1000/mm^3	144 (122–179)	136 (104–159)	0.001
Follow-up duration from baseline, median (IQR), years	–	5.0 (2.4–6.9)	–

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; IQR, interquartile range; IT, immune-tolerant; MA, mildly-active; SD, standard deviation; ULN, upper limit of normal range.
The ULN of ALT was defined as 30 IU/L for males and 19 IU/L for females.
*Laboratory test results were missing in 3 patients at HCC diagnosis.

Table 9. METAVIR fibrosis stage of the non-tumor liver tissues among the IT and MA phase patients who received surgery for HCC.

Characteristic	n = 45
Fibrosis (Stage), n (%)	
F1	5 (11.1%)
F2	9 (20.0%)
F3-F4	31 (68.9%)

Non-tumor liver histology was obtained in 45 patients who had either liver resection (n=42) or transplantation (n=3).

Figure 1. Patient flow chart

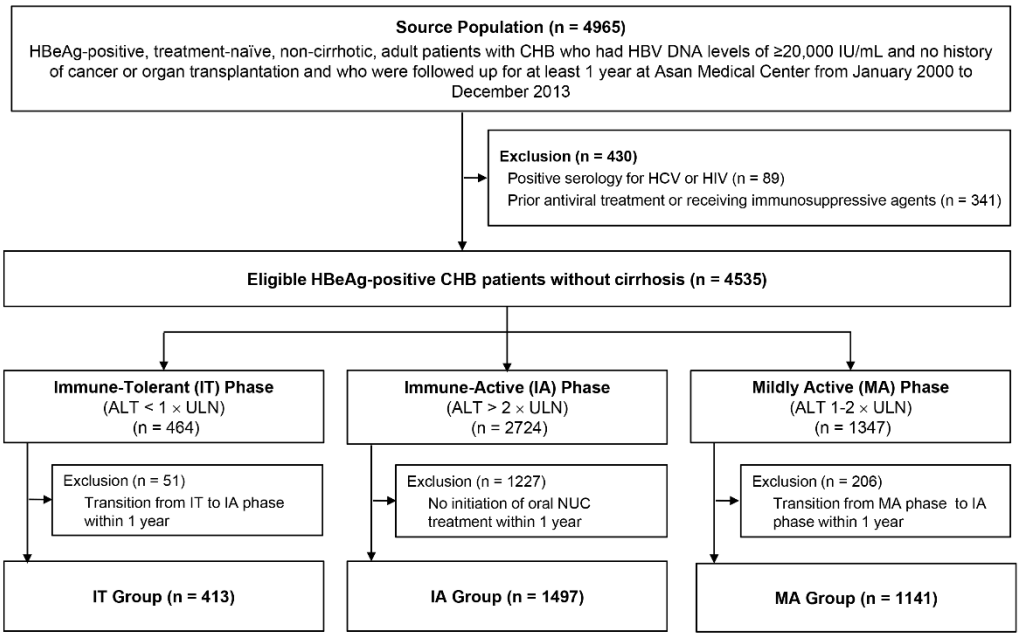


Figure 2. Cumulative incidences of hepatocellular carcinoma (HCC) and death or transplantation in the immune-tolerant vs. immune-active phase patients
(A) Cumulative incidence of HCC, (B) Cumulative incidence of death or transplantation

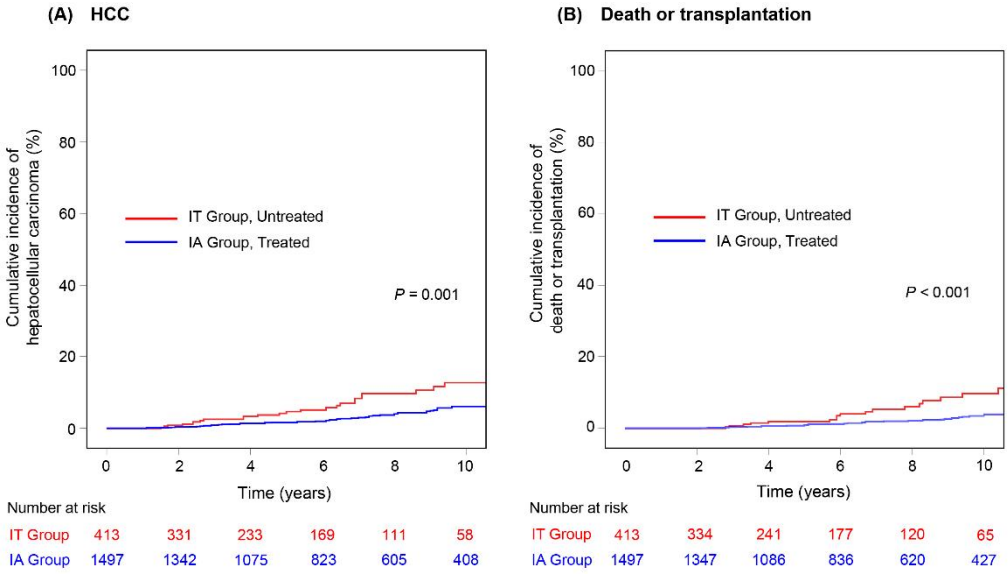


Figure 3. Propensity score-matched analysis for the cumulative incidences of HCC and death/transplantation in the immune-tolerant phase vs. immune-active phase patients.

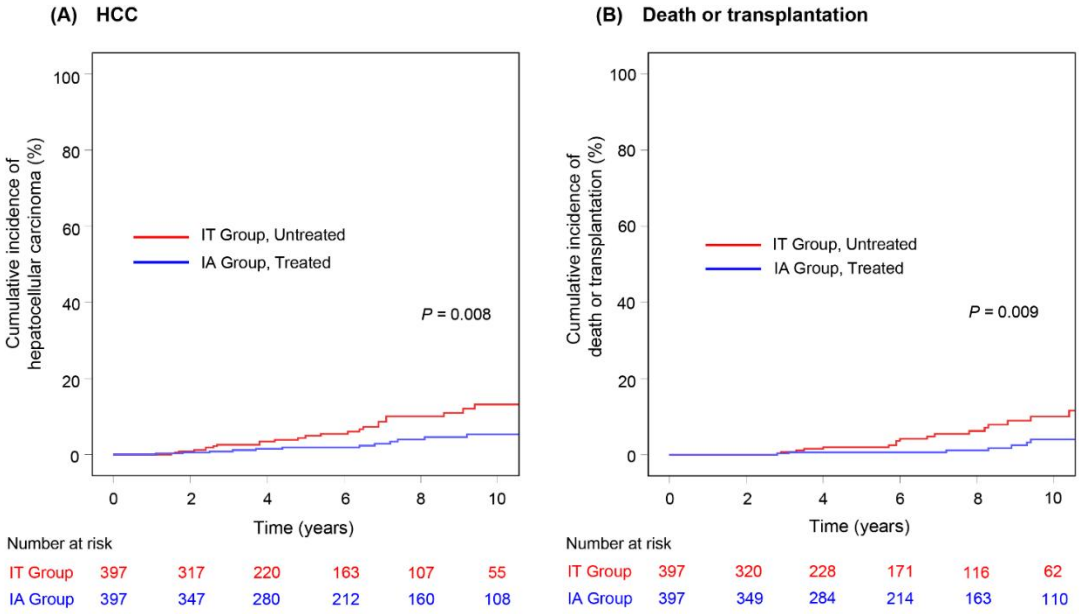


Figure 4. Cumulative incidences of hepatocellular carcinoma (HCC) and death or transplantation in the immune-tolerant vs. immune-active vs. mildly active phase patients
(A) Cumulative incidence of HCC. (B) Cumulative incidence of death or transplantation

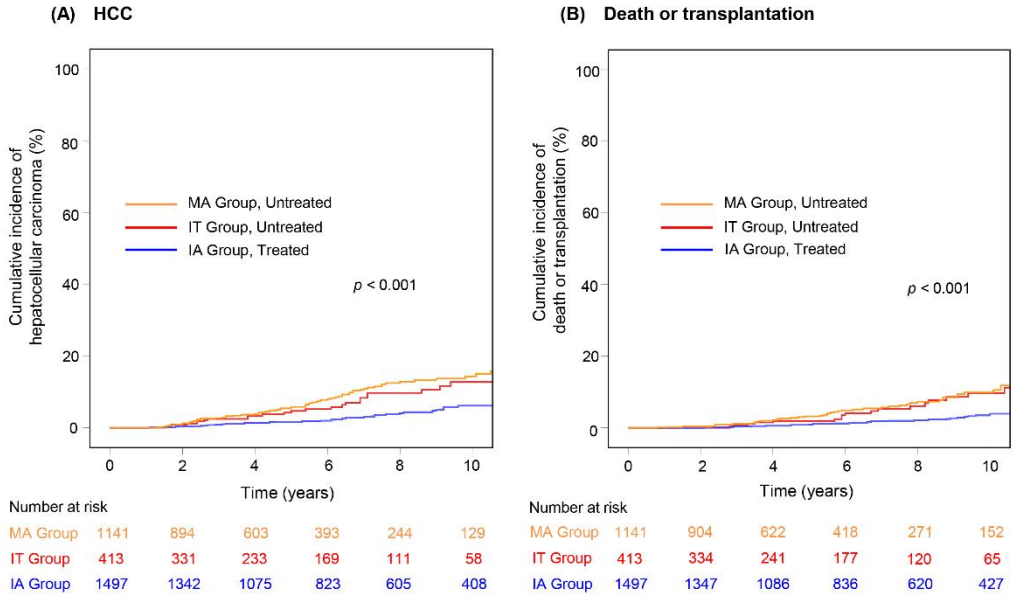


Figure 5. Cumulative incidence of clinical events (HCC, death, or transplantation) in the IT and MA groups vs. the IA group.

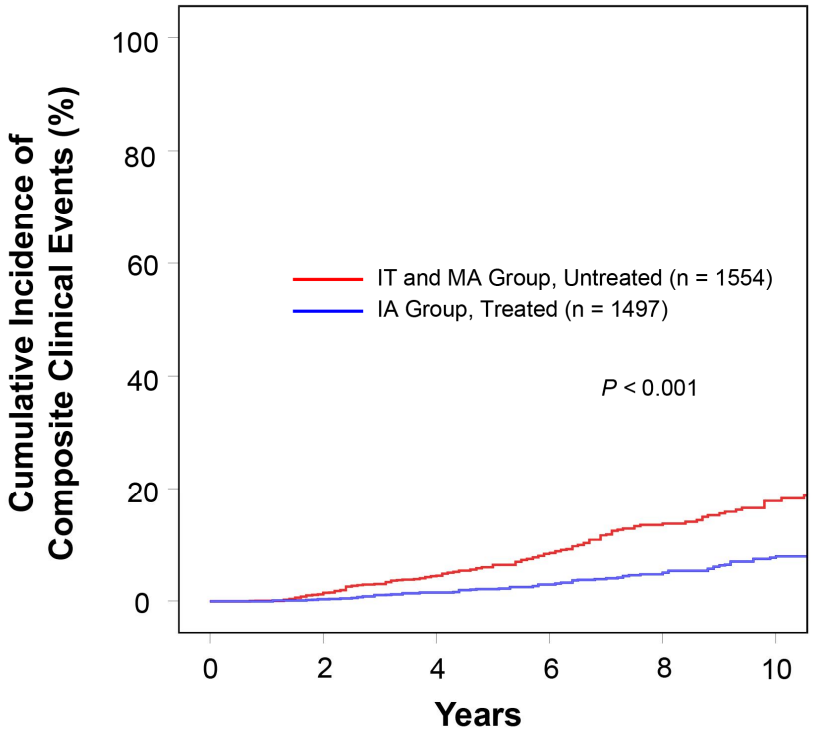
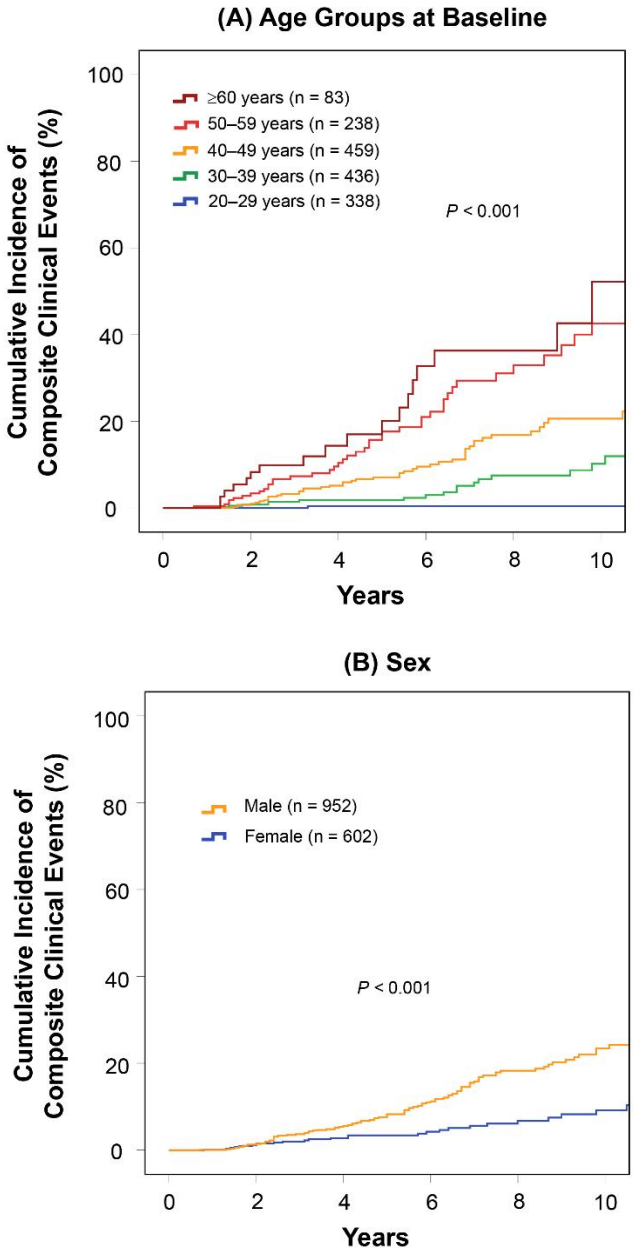


Figure 6. Factors predictive of clinical events (HCC, death, or transplantation) in the IT and MA groups.



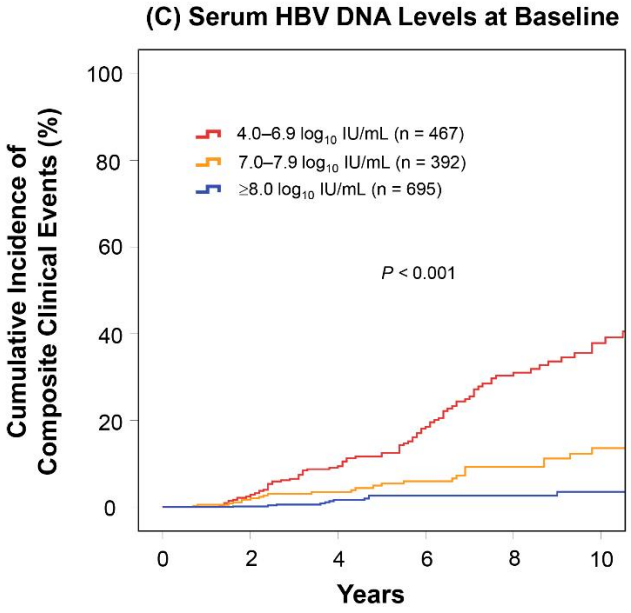

(C) Serum HBV DNA Levels at Baseline
Cumulative Incidence of Composite Clinical Events (%)
100
80
60
40
20
0
4.0–6.9 log10 IU/mL (n = 467)
7.0–7.9 log10 IU/mL (n = 392)
≥8.0 log10 IU/mL (n = 695)
P < 0.001
0
2
4
6
8
10
Years

Figure 7. Cumulative probability of the IT and MA phase patients to start nucleos(t)ide treatment due to transition to the IA phase by Kaplan–Meier analysis.