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Prevalence and Clinical Features of Patients with Concurrent HBsAg and Anti-HBs: Evaluation of the Hepatitis B Research Network Cohort

Running Title: **Concurrent HBsAg and Anti-HBs**

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The prevalence of concurrent HBsAg and anti-HBs in plasma of persons with chronic hepatitis B virus (HBV) infection is variable and its clinical significance enigmatic. We examined the prevalence,clinical and virological features of concurrent HBsAg and anti-HBs inchildren and adults with chronic HBV infection living in North America. A total of 1,462 HBsAg positive participants in the Hepatitis B Research Network pediatric and adult cohorts were included[median age 41 (range 4-80) years, 48% female, 11% white, 13% black, 73% Asians]. Only 18 (1.2%) were found to be anti-HBs positive (≥10 mIU/mL) at initial study evaluation.Distributions of sex, race, HBV genotype, and ALTwere similar between participants with and without concurrent anti-HBs. Those who were anti-HBs positive appeared to be older (median age 50 vs. 41 years,p=0.06), hadlower platelet counts (median 197 vs. 222 x 103/mm^3, p=0.07)and higher prevalence of HBeAg (44% vs. 26%, p=0.10). They also had lower HBsAg levels (median 2.0 vs. 3.5 $\log_{10}$ IU/mL, p=0.02).Testing of follow-up samples after a median of 4 years (range 1-6)in 12 of the 18 participants with initial concurrent anti-HBsshowed anti-HBs became undetectable in 6, decreased to <10 mIU/mL in 1and remained positive in 5 participants.Two patients lost HBsAg during follow-up. In conclusion, prevalenceof concurrent HBsAg and anti-HBs was low at 1.2%,with anti-HBs disappearing in some during follow-up, in this large cohort of racially diverse children and adults with chronic HBV infection living in North America. Presence of concurrent HBsAg and anti-HBs did not identify a specific phenotype of chronic hepatitis B, nor did it appear to affect clinical outcomes.

Introduction

The simultaneous presence in plasma of hepatitis B surface antigen (HBsAg) and antibody (anti-HBs) in patients with chronic hepatitis B virus (HBV) infection has long puzzled clinicians and investigators. The presence of both antigen and antibody typically results in immune complex formation and removal from the circulation(1).First reported in the 1970s, the most frequent explanation offered is that anti-HBs in this setting represents heterotypic antibodies not directed against the common 'a' determinant or the circulating HBV serotype(2-10). Alternatively, anti-HBs may not be neutralizing because of expression of variant HBsAg not recognized by anti-HBs (immune escape variant resulting from mutations in the S gene) (11-20).

Reported prevalence of concurrent HBsAg and anti-HBs has varied greatly, from 5-60%. Early United States (US) studies found concurrent HBsAg and anti-HBs present in 24% (64/269) and 32% (61/190) of patients, respectively(6,8), with a higher prevalence in patients with active hepatitis and hepatitis B e antigen (HBeAg)positivity (8). Larger recent studies have found lower (<10%) prevalence of concurrent HBsAg and anti-HBs(13-16, 19-23). In summary, existing literature indicatesa wide range in prevalence of concurrent HBsAg and anti-HBs in patients with chronic HBV infection and conflicting associations with activity of HBV replication and liver disease (6-23). Many of the early studies were limited by small numbers and possible selection bias, with little data on persistence of anti-HBs or HBsAg clearance. We sought to evaluate the prevalence, and clinical and virologic attributes of concurrent HBsAg and anti-HBs in a large sample of patients with chronic HBV infection participating in the Hepatitis B Research Network (HBRN) pediatric and adult cohortstudies. A secondary aim was to evaluate the stability of concurrent HBsAg and anti-HBs over time.

Methods

Study design

The HBRN is a clinical research network, funded by the National Institute for Diabetes and Digestive and Kidney Diseases at the National Institutes of Health, that enrolled patients with HBV infection into pediatric and adult cohortstudies from 2012-2017.Details of the HBRN cohort study protocols have been previously described (24,25). All 21 adult and 7 pediatric sites in North Americaenrolled HBsAg positive patients, not currently receiving antiviral therapy, with no history of hepatic decompensation, hepatocellular carcinoma, liver transplant, or known HIV infection.

Participant evaluations included a medical history, a brief physical examination, completion of questionnaires,standard of care tests, and collection of blood samples for research-related testing.In addition, relevant clinical, laboratory, radiological, and histological data werecollected from participants' medical records.After enrollment, adult participants were seen at week 12, 24 and then every 24 weeks (i.e., bi-annually); and pediatric participants at week 24, 48 and then every 48 weeks (i.e., annually).

The protocols governing this research were approved by the institutional review boards of each participating institution and each participant gave written, informed consent for his/her participation.In the case of minors, written informed consent was given by their parent or guardian and, where possible, the children themselves gave assent to participate.All authors had access to the study data and reviewed and approved the final manuscript.

Central HBV testing

Qualitative and quantitative HBsAg, quantitative HBeAg, and quantitative HBV DNA testing were performed on samples from annual assessments at the University of Washington.Quantitative HBeAg and HBV DNA were also tested with samples from bi-annual assessments among adults.HBV DNA levels were determined using a real-time PCR assay (COBAS Ampliprep/COBAS TaqMan HBV Test, v2.0; Roche Molecular Diagnostics, Branchburg, NJ) with a lower limit of detection (LLOD) of 10 IU/mL and a lower limit of quantification (LLOQ) of 20 IU/mL.Quantitative HBeAg and HBsAg were tested using the Roche Diagnostics Elecsys platform with LLOD and LLOQ of 0.3 IU/mL for HBeAg and 0.05 IU/mL for HBsAg.HBsAg and HBV DNA were log transformed (log_{10}) for analysis. HBV genotype was determinedusing automated mass spectrometry at the Molecular Epidemiology and Bioinformatics Laboratory in the Division of Viral Hepatitis at the Centers for Disease Control and Prevention (26).

Baseline determination of quantitative anti-HBs testing was added to the HBRN cohort study protocols in 2018 because anti-HBs testing had not been consistently done at local sites.Quantitative anti-HBs testing was performed at the University of Texas Southwestern using an enzyme-linked immunosorbent assay (ELISA) (Diasorin Inc., Stillwater, MN, USA);the

LLOQ was 5 mIU/mL and ≥10 mIU/mL was considered positive (18). The first available sample of HBRN participants with chronic HBV infection,and no co-infection (HCV, HDV or HIV), collected up to 2 yearspast enrollment was used to determine baseline prevalence of concurrent HBsAg and anti-HBs, defined as positive HBsAg and anti-HBs ≥10 mIU/mL.Samples collected while participants were on HBV treatment or pregnant were excluded. Among those who were anti-HBs positive at baseline, blood samples collected from an assessment at least one yearafter the first sample and at participants' last assessment (if at least 3 years from the first sample and at least1 year from the second sample) were also tested for quantitative anti-HBs regardless of treatment status to determine persistence of anti-HBs.

Statistical analyses

Statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA). Reported p-values are two-sidedand were used to guide interpretation of results (27). The Chi-Square test, Fisher-exact test and the Wilcoxon-Rank Sum test, as appropriate, were used to test for associations between demographic, clinical and virological features and concurrent HBsAg and anti-HBs.Box plots were used to visualize the distribution of quantitative HBsAg and HBV DNA levels in participants with and without concurrent anti-HBs. Scatter plots were used to visualize the distribution of anti-HBs levels in relation to quantitative HBsAg and HBV DNA levels, and Pearson correlation coefficients are reported.

Results

A total of 2,340adult and pediatric participants with chronic HBVinfection and no HCC or HIV co-infection had enrolled in the HBRN study at the time samples for this ancillary study were selected.Of these,971 participants were excluded due toinsufficientstored research blood sampleswithin 2 years of enrollment. The remaining1462 participants were included in this analysis. Of these, anti-HBs testing was performed using serum drawn at enrollment in 1263 (86.4%) participants and between enrollment and year 2 in the remaining participants.

Of the 1,462 (124 children and 1,338 adults) participants tested for anti-HBs, 18 (1.2%) tested positive [1/123 (0.8%) children and 17/1321 (1.3%) adults]. An additional 13 (0.9%) participants had detectable anti-HBs but levels were <10 mIU/mL and were considered to be anti-HBs negative. **Table 1** compares the demographics, clinical and virologic features of the participants with and without concurrent anti-HBs. The two groups had similar distributions of sex, race, and place of birth but the anti-HBs positive group appeared to be older (median age 49.8 vs. 41.3 years; p=0.06).

Clinical and virologic features

The two groups were similar in prevalence of hepatitis C or D co-infections, distribution of HBV genotypes, family history of HBV infection, and prior HBV treatment, as well as median serum aspartate and alanine aminotransferase (AST, ALT) levels(**Table 1**).HBsAg levels were lower in those with concurrent anti-HBs (median 2.0 vs. 3.5 log10 IU/mL, p=0.02;**Figure S1A**), along with somewhat higher serum HBV DNA levels (median 4.9 vs. 3.7 log10 IU/mL, p=0.20;**Figure S1B**),although one(5.6%) had undetectable serum HBV DNA (case 6; **Table 2**).ALT levels were not significantly different between groups (1.3 vs. 1.4, p=0.55; **Figure S1C).**

Anti-HBs levels were below 50 mIU/mL in nearly all(15 of 18)concurrent anti-HBs participants, the highest level being 95 mIU/mL. Among participants with concurrent anti-HBs, correlations between anti-HBs levels and HBsAg (r=0.35, p=0.16) and HBV DNA levels (r=0.41, p=0.09) were weak (**Figure S2A and 2B**).

Persistence of anti-HBs

Among the 18 participants with concurrent anti-HBs at baseline, 12 had stored samples for one follow-up and 10 for two follow-uptests. Of these 12,anti-HBs levels remained ≥10 mIU/mL in 5 participants(cases 1-5), decreased to <10 mIU/mL but remained detectable in 1 participant (case 6), and became undetectable in 6 participants(cases 7-12), (**Table 2**).Three of 6 in whom anti-HBs became undetectable had started HBV treatment first (case 7, 10, 12).

Figures with viral markers across time for the 12 participants with follow-up anti-HBs data are provided in supplemental material (**Figure S3**). In general, HBsAg levels were largely unchangedduring follow-up.All 6 participants with decreases in HBV DNA were on HBV treatment (**Table 2** and **Figure S3**).

Outcomes:HBsAg loss, hepatic decompensation and hepatocellular carcinoma

During a median follow-up of 5.5 years (range 1.2-6.4), 2 of the 12 participants with concurrent anti-HBs at baseline and follow-up HBsAg data becameHBsAg negative(cases3and

6). One participant (case 6) first became HBsAg negative 3.5 years after baseline. The other participant (case 3) first became HBsAg negative 4.1 years after baseline but HBsAg was transiently positive again at low level (2 IU/mL) at 4.9 years when anti-HBs was positive at 41.3 mIU/mL(**Table 2**).

None of the 14participants with concurrent anti-HBs at baseline who returned for follow-up developed hepatic decompensation or hepatocellular carcinoma.

Discussion

This large study provided a unique opportunity to assess prevalence and durability of concurrent HBsAg and anti-HBs among persons with chronic HBV infection in North America. The very low overall prevalence of 1.2%, 0.8% in children and 1.3% in adults, islower than the two US adult studies from the 1980s(6,8). Reasons for these differences include ourexclusion of thosereceiving antiviral therapy at enrollment; as a result, most participants had low level HBV replication and inactive liver disease. Notably, we did not observe differences in AST, ALT or HBV DNA levels between participants with and without concurrent anti-HBs. Second, exclusion of patients on treatment also virtually eliminated patients with cirrhosis, whomight be more likely to have concurrent anti-HBs.Third, we used anti-HBs level ≥10 mIU/mL to define positive anti-HBs, whereas the previous US studies used any detectable level anti-HBs. However, even if we had included the 13 participants with detectable anti-HBs (i.e.,≥5mIU/mL) but levels <10 mIU/mL, the prevalence of concurrent anti-HBs would only be 2.1%.

Our study's low prevalence of concurrent HBsAg and anti-HBs is consistent withseveral more recent large studies including ≥1,000 participants each:using anti-HBs ≥10 mIU/mL as cutoff; most of these studies showed a prevalence ≤5% (**Table3**).

We did not find an association of concurrent HBsAg and anti-HBs with sex, race, country of birth, HBV genotype or ALT level, a finding similar to the majority of studies published since 1980 (**Table 3**).Of thosestudies that examined associations with demographics, none reported an association with age or sex (10, 15, 16, 19, 20, 27, 28). While several previous studies, like ours, found an association between concurrent HBsAg and anti-HBs positivity and HBeAg positivity (8, 28, 17),more did not (9, 10,14,15, 18, 19, 20, 21, 27).Likewise, most prior studies, like ours, reported no association with ALT (10, 14, 5, 16, 17, 19, 20, 27, 28).

While prior studies suggested no specific pattern of HBsAg levels(10,14,16,18,19), we found a marked difference in HBsAg levels between participants with and without concomitant anti-HBs (median 2.0 vs. 3.5 log10 IU/mL). We hypothesize that the presence of anti-HBs may partially neutralize or bind to circulating HBsAg accounting for the lower HBsAg levels. In general, anti-HBs levels were quite low (range 10-95 mIU/mL), with a wide range in HBsAg levels (8-27,090 IU/mL) at baseline in the 18 participants with concurrent anti-HBs. Although there was no overall correlation between anti-HBs and HBsAg or HBV DNA, when the groups were separated into HBeAg positive and negative, a moderate correlation for the HBeAg positive subjects was apparent between anti-HBs and both HBsAg and HBV DNA titers (s**Figure 2**).In summary, our data are in line with most published studies, showing lower anti-HBs levels in patients with concurrent HBsAg and anti-HBs, and no consistent association of HBV DNA levels to presence of concurrent HBsAg and anti-HBs(**Table S1**).

Our study included children,diverse races and HBV genotypes, as well as provideddata on durability of concurrent anti-HBs,using a central lab for the anti-HBs measurements. However, the small number of participants with concurrent HBsAg and anti-HBs limited our statistical power for all analyses and our ability to examine the clinical significance of concurrent HBsAg and anti-HBs. Further limitations include the lack of HBV sequencing and HBsAg and anti-HBs serotyping data in the 18 participants with concurrent HBsAg and anti-HBs.

In summary, we found a low prevalence of concurrent anti-HBs in this large cohort of racially diverse children and adults with chronic HBV infection living in North America. Our prevalence estimate contrasts with two much earlier US studies but resembles contemporary studies from Asia and Europe. There being no association with HBV replication or liver disease or signs of immune complex disease, there appear to be no important clinical implications to anti-HBs positivity in patients with chronic HBV infection. Anti-HBs levels were uniformly low and became undetectable in half during a median follow-up of 4 years.Concurrent HBsAg and anti-HBs appears to have no clinical significance in regard to viral clearance or disease resolution; thus, patients with chronic HBV infection who are anti-HBs positive should be managed similarly to those who are anti-HBs negative.

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Supplementary Figure Legends

SupplementaryFigure 1. Box plot showing HBsAg level (A), HBV DNA level (B), and ALT level (C) among children and adults with chronic HBV infection by anti-HBs status.

SupplementaryFigure 2. Scatter plots of anti-HBs level by HBsAg level (A) and HBV DNA level (B) at baseline in children and adults with chronic HBV with concurrent HBsAg and anti-HBs. HBeAg status indicated.

Abbreviations: HBsAg, hepatitis B surface antigen; anti-HBs, hepatitis B surface antibody

There were significant positive correlations between anti-HBs levels and HBsAg and DNA (r=0.73, p=0.04; r=0.74, p=0.03, respectively), among HBeAg positive participants only.

SupplementaryFigure 3. Anti-HBs, HBsAg, HBV DNA and levels over time in participants with chronic HBV infection and concurrent anti-HBs and HBsAg at baseline. No participants were receiving HBV treatment (Rx) at baseline. Among participants who initiated treatment during follow-up, the start and stop of treatment is indicated.

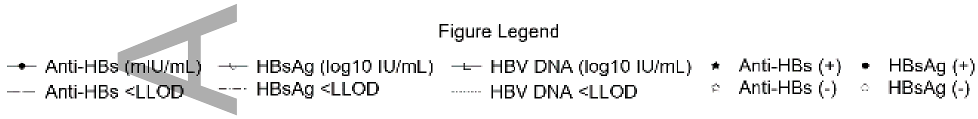

Table 1. Demographics, clinical and virologic characteristics among HBsAg positive children and adults with chronic HBV infection, and anti-HBs status[a] by demographic, clinical and virologic characteristics

Characteristic	Total N=1462	Anti-HBs- N=1444[b]	Anti-HBs+ N=18[b]	p-value
Age, years				0.06
Median(25th, 75th)	41.3 (31.3, 52.0)	41.3 (31.3, 51.9)	49.8 (36.3, 63.7)	
Min, Max	4.0, 80.2	4.0, 80.2	16.1, 75.3	
Age, years				0.23
<18	124 (8.5)	123 (99.2)	1 (0.8)	
18-<30	203 (13.9)	201 (99.0)	2 (1.0)	
30-<40	345 (23.6)	341 (98.8)	4 (1.2)	
40-<50	355 (24.3)	353 (99.4)	2 (0.6)	
≥50	435 (29.8)	426 (97.9)	9 (2.1)	
Gender, n (%)				0.51
Male	763 (52.2%)	755 (99.0%)	8 (1.0%)	
Female	699 (47.8%)	689 (98.6%)	10 (1.4%)	
Race, n (%)	n=1458	n=1440		0.95
White	164 (11.2%)	163 (99.4%)	1 (0.6%)	
Black	189 (13.0%)	187 (98.9%)	2 (1.1%)	
Asian	1066 (73.1%)	1051 (98.6%)	15 (1.4%)	
Other	39 (2.7%)	39 (100.0%)	0 (0.0%)	
Place of birth, n (%)	n=1460	n=1442		0.87
United States/Canada	269 (18.4%)	267 (99.3%)	2 (0.7%)	
Other North America/South America	21 (1.4%)	21 (100.0%)	0 (0.0%)	
Europe	53 (3.6%)	53 (100.0%)	0 (0.0%)	
Asia/Australia	987 (67.6%)	972 (98.5%)	15 (1.5%)	
Africa	130 (8.9%)	129 (99.2%)	1 (0.8%)	
HDV+				0.42
No	1419 (97.1%)	1402 (98.8%)	17 (1.2%)	
Yes	43 (2.9%)	42 (97.7%)	1 (2.3%)	
HCV+				0.99
No	1437 (98.3%)	1419 (98.7%)	18 (1.3%)	
Yes	25 (1.7%)	25 (100.0%)	0 (0.0%)	
Known family history of chronic HBV, n (%)	n=1137	n=1124	n=13	0.99
No	410 (36.1%)	405 (98.8%)	5 (1.2%)	
Yes	727 (63.9%)	719 (98.9%)	8 (1.1%)	

Prior HBV treatment, n (%)				0.73
No	1256 (85.9%)	1241 (98.8%)	15 (1.2%)	
Yes	206 (14.1%)	203 (98.5%)	3 (1.5%)	
HBeAg, n (%)	n=1459	n=1441		0.10
Negative	1074 (73.6%)	1064 (99.1%)	10 (0.9%)	
Positive	385 (26.4%)	377 (97.9%)	8 (2.1%)	
HBsAg (log_{10} IU/mL)	n=1393	n=1376	n=17	0.02
Median(25th:75th)	3.5 (2.7: 4.2)	3.5 (2.7: 4.2)	2.0 (1.7: 4.2)	
HBV DNA (log_{10} IU/mL)	n=1461	n=1443		0.20
Median(25th:75th)	3.7 (2.6: 6.0)	3.7 (2.6: 6.0)	4.9 (3.4: 5.8)	
Genotype, n (%)	n=1359	n=1342	n=17	0.57
A-1	92 (6.8%)	92 (100.0%)	0 (0.0%)	
A-2	126 (9.3%)	125 (99.2%)	1 (0.8%)	
B	530 (39.0%)	522 (98.5%)	8 (1.5%)	
C	434 (31.9%)	427 (98.4%)	7 (1.6%)	
D	127 (9.3%)	127 (100.0%)	0 (0.0%)	
E	36 (2.6%)	35 (97.2%)	1 (2.8%)	
Other	14 (1.0%)	14 (100.0%)	0 (0.0%)	
ALT xULN[c]	n=1426	n=1409	n=17	0.55
Median(25th:75th)	1.3 (0.9: 2.0)	1.3 (0.9: 2.0)	1.4 (1.0: 2.7)	
AST xULN[d]	n=1404	n=1387	n=17	0.67
Median(25th:75th)	0.7 (0.6: 1.0)	0.7 (0.6: 1.0)	0.8 (0.6: 1.1)	
Platelets ($x10^3/mm^3$)	n=1264	n=1250	n=14	0.07
Median(25th:75th)	221 (183.5: 259)	221.5 (184: 259)	196.5 (167: 223)	

Abbreviations: ALT, Alanine aminotransferase; DNA, deoxyribonucleic acid; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HCV, Hepatitis C virus; HDV, Hepatitis D virus, ULN, upper limit of the normal range.

[a]Anti-HBs+ was defined as anti-HBs ≥10 mIU/mL.

[b]Data are reported among this sample unless a smaller number is indicated due to missing data.

[c]The ULN for ALT was standardized based on sex and age (i.e., ≤33 U/L for males and females ages < 1 year; ≤25 U/L for males and females ages 1 year-<13 years; ≤25 U/L for males and ≤22 U/L for females ages 13 years-<18 years; ≤30 U/L for adult males and ≤20 U/L for adult females).

[d]The ULN for AST was based on lab-specific ULN

Table 2. Anti-HBs, HBsAg, qualitative HBeAg and HBV DNA levels over time in participants with chronic HBV infection and concurrent HBsAg and anti-HBs at baseline[a]

Case no.	Years since baseline	Age, years	Phenotype[f]	Anti-HBs (mIU/mL)	HBsAg (log_{10} IU/mL)	HBeAg qualitative	HBV DNA (log_{10} IU/mL)	ALT x ULN
1	0.0	27	Inactive carrier	10.0	3.6	Negative	3.7	0.5
	1.1	28	Inactive carrier	.	3.6	Negative	3.6	0.6
	1.9	29	Inactive carrier	12.6	3.5	Negative	3.5	0.5
	2.9	30	Inactive carrier	10.0	3.5	Negative	3.5	0.6
2	0.0	39	Inactive carrier	10.3	4.3	Negative	3.8	0.9
	1.0	40	Indeterminate	29.1	4.3	Negative	3.7	2.1
	2.3	42	Indeterminate	.	.	Negative	3.6	1.2
	2.9	42	Indeterminate	18.2	4.2	Negative	3.6	1.2
	4.8	44	Indeterminate	.	4.2	Negative	3.4	1.3
3	0.0	69	HBeAg+ CHB	11.0	1.3	Positive	5.5	1.0
	1.0	70	Unknown	.	1.3	Positive	5.8	.
	2.0	71	Immune tolerant	15.0	1.4	Positive	5.4	0.9
	2.7	72	Immune tolerant	.	0.2	Positive	5.3	0.9
	3.5	73	Immune tolerant	.	1.0	Positive	5.2	1.2
	4.9	74	Immune tolerant	41.3	.02	Positive	5.2	0.9
	5.6	75	HBsAg negative	.	Negative[b]	.	5.5	0.9
	6.2	76	HBsAg negative	.	.	Positive	5.1	1.1
4	0.0	36	HBeAg+ CHB	112.9	4.5	Positive	8.1	3.0
	0.9	37	HBeAg+ CHB	103.1	4.5	Positive	8.1	1.4
	1.4	37	HBeAg+ CHB	.	.	Positive	8.3	1.1
5	0.0	33	HBeAg+ CHB	94.7	4.4	Positive	8.1	1.6
	1.0	34	HBeAg+ CHB	156.6	4.5	Positive	8.1	3.0
	6.4	39	HBsAg negative	88.6	.	.	<LLOQ[c]	0.6
6	0.0	72	Indeterminate	13.1	Positive[b]	Negative	<LLOD	1.4
	1.0	73	Indeterminate	9.6	Positive[b]	Negative	<LLOD	1.1
	2.0	74	Indeterminate	.	Positive[b]	.	.	1.2
	3.0	75	Indeterminate	.	.	.	<LLOQ	1.3
	4.5	76	HBsAg negative	.	Negative[b]	.	<LLOQ	0.6
	5.5	77	HBsAg negative	.	Negative[b]	.	<LLOQ	0.9
7	0.0	57	HBeAg+ CHB	15.9	1.8	Positive	5.8	2.5
	0.8	57	HBV med	.	1.5	Positive	2.2[c]	1.7
	1.8	58	HBV med	<LLOD	1.6	Negative	<LLOD	0.9
	2.7	59	HBV med	<LLOD	1.6	Negative	<LLOQ	0.8
	3.7	60	HBV med	.	.	.	<LLOQ	.
	5.2	62	HBV med	.	.	.	<LLOQ	.
8	0.0	26	HBeAg+ CHB	40.6	4.3	Positive	8.3	3.0
	0.9	27	HBeAg+ CHB	.	4.3	Positive	8.2	2.2
	1.8	28	HBeAg+ CHB	16.6	4.1	Positive	8.2	2.9

	2.7	29	HBeAg+ CHB	.	4.2	Positive	8.4	3.4
	3.6	30	HBeAg+ CHB	.	4.2	Positive	8.2	4.0
	4.6	31	HBeAg+ CHB	<LLOD	4.1	Positive	8.2	11.6
	5.5	32	HBeAg+ CHB	.	Positive[b]	Positive	8.0	13.9
9	0.0	60	Indeterminate	10.9	2.0	Negative	3.1	1.2
	0.8	61	Indeterminate	.	2.0	Negative	3.7	1.1
	1.8	62	Inactive carrier	.	1.9	Negative	1.6	0.9
	2.8	63	Inactive carrier	<LLOD	1.9	Negative	2.0	1.0
	3.8	64	HBV med	.	1.8	Negative	<LLOD[c]	1.6
	4.6	65	HBV med	.	1.7	Negative	<LLOD	0.8
	5.5	66	Inactive carrier	<LLOD	1.5	Negative	<LLOD	0.9
	6.3	67	Inactive carrier	.	.	.	<LLOQ	0.9
10	0.0	75	HBeAg- CHB	17.3	1.8	Negative	5.0	2.9
	0.9	76	HBV med	.	2.1	Negative	<LLOD[c]	0.8
	1.8	77	HBV med	<LLOD	2.2	Negative	<LLOD	1.0
	2.7	78	HBV med	.	2.1	Negative	<LLOD	1.1
	3.6	78	HBV med	.	2.1	Negative	<LLOD	1.1
	4.6	79	HBV med	.	2.0	Negative	<LLOD	1.2
	5.5	80	HBV med	<LLOD	2.0	Negative	<LLOD	1.1
	6.4	81	HBV med	.	Positive[b]	.	<LLOQ	1.1
11	0.0	49	Indeterminate	11.2	2.0	Negative	3.4	2.8
	1.0	50	Indeterminate	.	2.0	Negative	4.2	1.0
	1.9	51	HBeAg- CHB	8.8	1.9	Negative	4.1	1.3
	2.8	51	Indeterminate	.	1.8	Negative	3.7	1.7
	3.8	52	HBV med	.	1.8	Negative	<LLOQ[c]	1.7
	4.6	53	HBV med	.	.	Negative	<LLOQ	3.4
	5.6	54	HBV med	<LLOD	1.9	Negative	<LLOQ	1.6
	6.5	55	HBV med	.	.	.	<LLOQ	1.9
12	0.0	63	Indeterminate	12.0	0.9	Positive	4.7	1.1
	1.0	64	Indeterminate	.	1.0	Positive	4.7	1.6
	1.4	65	HBV med	9.9	.	Positive	2.9[c]	1.2
	1.8	65	HBV med	.	1.5	Positive	1.4	1.2
	2.8	66	HBV med	.	2.2	Positive	1.5	1.2
	3.6	67	HBV med	<LLOD	2.7	Positive	<LLOQ	1.0
	4.6	68	HBV med	.	3.0	Positive	<LLOQ	0.9
	5.5	69	HBV med	.	Positive[b]	Positive	<LLOQ	1.0
13[d]	0.0	67	HBeAg+ CHB	34.1	1.7	Positive	5.7	2.1
	0.6	68	HBV med	.	.	.	1.6[c]	1.1
14[d]	0.0	45	HBeAg- CHB	27.1	2.7	Negative	5.6	2.7
	5.3	50	HBV med	.	.	.	3.1[c]	2.8
15[e]	0.0	56	Indeterminate	40.9	1.4	Negative	2.2	1.2
16[e]	0.3	36	Indeterminate	132.8	2.2	Negative	4.3	0.9
17[e]	0.0	50	Inactive carrier	25.1	1.7	Negative	2.7	0.6
18[e]	0.0	16	HBV med	23.8	4.2	Positive	7.2	.

Abbreviations: ALT, alanine aminotransferase; DNA, deoxyribonucleic acid; HBsAg, hepatitis B surface antigen; LLOD = lower limit of detection, LLOQ = lower limit of quantification.

LLOD and LLOQ were 5.0 mIU/mL for anti-HBs, 0.05 IU/mL for HBsAg, 10 and 20 IU/mL, respectively, for HBV DNA.

[a]Baseline refers to time point when concurrent presence of HBsAg and anti-HBs was first determined; none of the participants were receiving HBV treatment at that time.

[b]Local laboratory results are reported when central laboratory testing was not performed. Only qualitative HBsAg testing was done at local laboratories. No participants were HBsAg negative by central laboratory qualitative testing.
[c]Started HBV treatment prior to this assessment.
[d]Cases 13-18 did not have stored samples available for follow-up anti-HBs testing.
[e]Cases 15-18 did not return for follow-up.
[f]Phenotype was determined from HBeAg status, ALT and HBV DNA among HBsAg positive participants not taking HBV medication. Immune tolerant HBV infection was defined by the presence of normal serum ALT levels despite the presence of HBeAg and HBV DNA $\geq 10^5$ IU/mL. The diagnosis of "immune active" chronic hepatitis B was based on the presence of elevated ALT levels accompanied by high levels of HBV DNA (i.e., $\geq 10^5$ IU/mL for HBeAg-positive and $>10^4$ IU/mL for HBeAg-negative patients). The inactive carrier state was defined by the presence of normal ALT levels and the absence of HBeAg with HBV DNA $\leq 10^4$ IU/mL. Participants who did not fulfil criteria for one of these categories were categorized as having an "indeterminant" phenotype: HBeAg positive and low HBV DNA $< 1 \times 10^5$ IU/ml was indeterminate A, HBeAg negative, elevated ALT and HBV DNA $\leq 10^4$ IU/mL was indeterminate B, and HBeAg negative, normal ALT and HBV DNA $>10^4$ IU/mL was indeterminate B.

Table 3. Prevalence of concurrent HBsAg and anti-HBs and associated factors in published studies of adults.

Author/yr (ref)	Country	Anti-HBs cutoff, mIU/mL	Anti-HBs+, n/N (%)	Factors associated with anti-HBs+	Associations not supported
Heijtink/1982 (6)	Netherlands	NR	32/89 (36.0)	Advanced liver disease	Risk factors for HBV
Tsang/1986 (7)	US	NR	64/269 (23.9)	NR	NR
Shiels/1987 (8)	US	NR	60/190 (31.6)	HBeAg+, active liver disease	Risk factors for HBV, presence of HDV
Hayashi/1990 (9)	Japan	NR	166/638 (26.1)	None	HBeAg, liver damage
Wang/1996 (21)	Singapore	All positive >10	234/1132 (21.0) 80/1132 (7.1)	None	HBeAg, HBV DNA
Lada/2006[a] (12)	France	NR	77/866 (8.9)	NR	NR
Zhang/2007 (10)	China	NR	20/411 (4.9)	None	Sex, age, ALT HBeAg, HBV DNA
Colson/2007[a] (11)	France	>10	13/459 (2.8)	Lower HBV DNA level	
Jang/2009 (29)	Korea	>10, confirmed on repeat test after 6 months	48/755 (6.4)	HBeAg+, HCC	Sex, age, ALT HBV DNA
Huang/2010 (13)	China	>10	34/1000 (3.4)	None	HBV DNA
Chen/2011[a] (14)	China	>10	72/1985 (3.6)	Lower HBsAg level, lower HBV DNA level	HBeAg, ALT
Liu/2012[a] (15)	China	>10	54/1862 (2.9)	None	Sex, age, ALT, HBeAg
Lee/2013 (22)	Korea	>10	353/177,954 (2.9)	Higher AST and ALT	NA
Seo/2014 (28)	Korea	NR	73/1042 (7.0)	Higher incidence of HCC on follow-up	Sex, age, ALT, cirrhosis, HBeAg, HBV DNA
Ding/2015[a] (16)	China	>10	39/1606 (9.8)	None	Sex, age, ALT, HBV DNA, HBV genotype, HBsAg level
Pancher/2015 (23)	France	>10	129/2578 (5.0)	NR	NR
Pu/2016 (17)	China	NR	122/4169 (2.9)	Older, HBeAg+, higher HBV DNA level	Sex, ALT
Liu/2016a (18)	China	≥10	436/13080 (3.3)	Lower ALT,	HBeAg

				lower HBsAg level, lower HBV DNA level	
Fu/2017[a] (19)	China	>10	145/5513 (2.6)	Lower HBsAg level, Lower HBV DNA level	Sex, age, ALT, HBeAg
Xiang/2017 (30)	China	NR	324/124,865 (0.3)	NR	NR
Liu/2018[a] (20)	China	>10	179/4455 (4.0)		Sex, age, ALT, HBeAg, HBV DNA
Current study	US	>10	18/1462 (1.2)	Older age, lower platelets, HBeAg+, lower HBsAg level	Sex, race, ALT, , HBV genotype, HBV DNA level, HCV or HDV infection

Abbreviations: ALT, Alanine aminotransferase; DNA, deoxyribonucleic acid; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HCV, Hepatitis C virus; HDV, Hepatitis D virus; NR, not reported.

[a]Comparisons of persons with concurrent HBsAg and anti-HBs made with subset of controls.