Supplemental Material for Comparison of Novel Biomarkers with Conventional HBV Markers among Untreated Adults with Chronic Hepatitis B in North America

Figure S1: Consort diagram of derivation of study cohort.

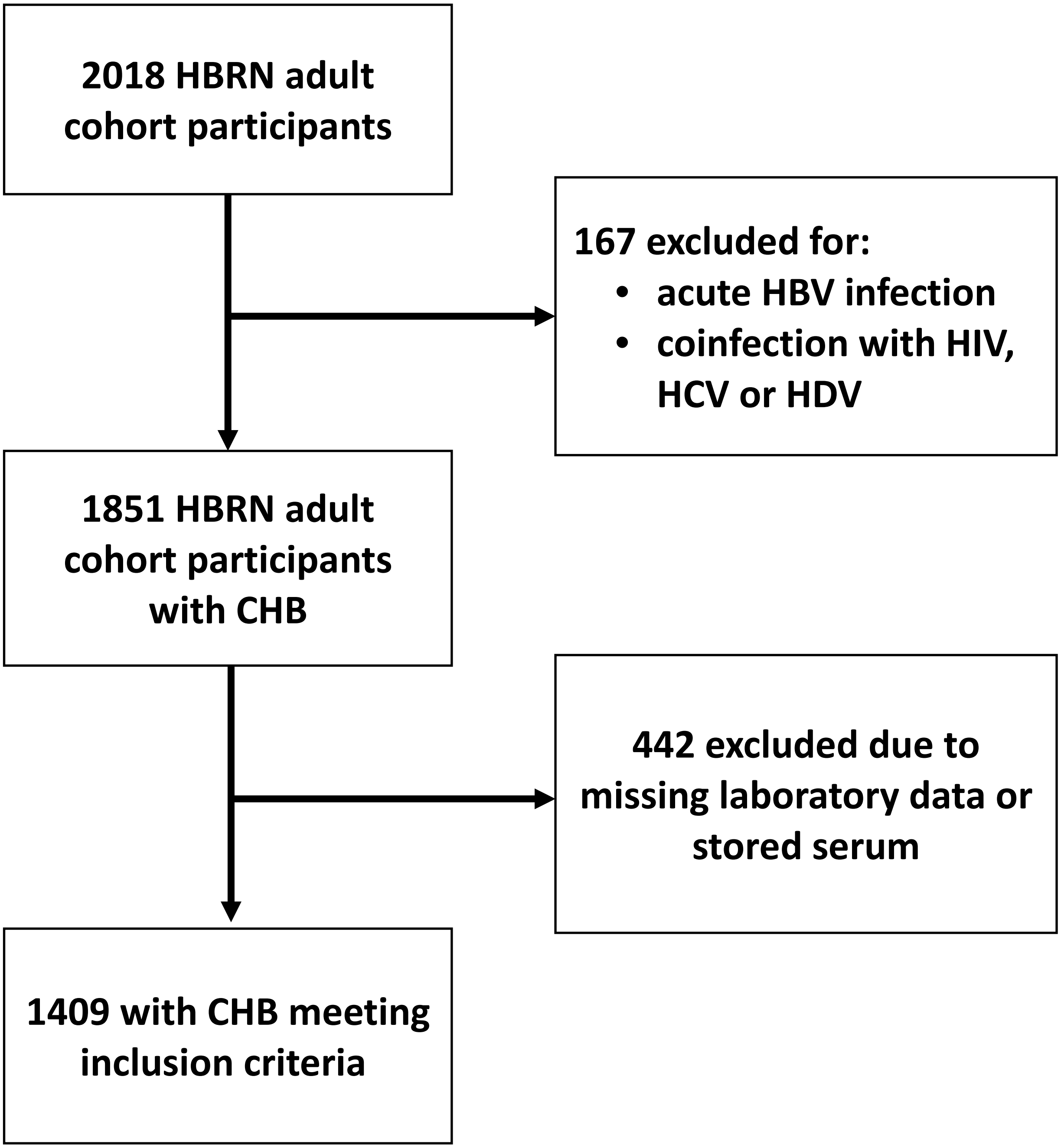
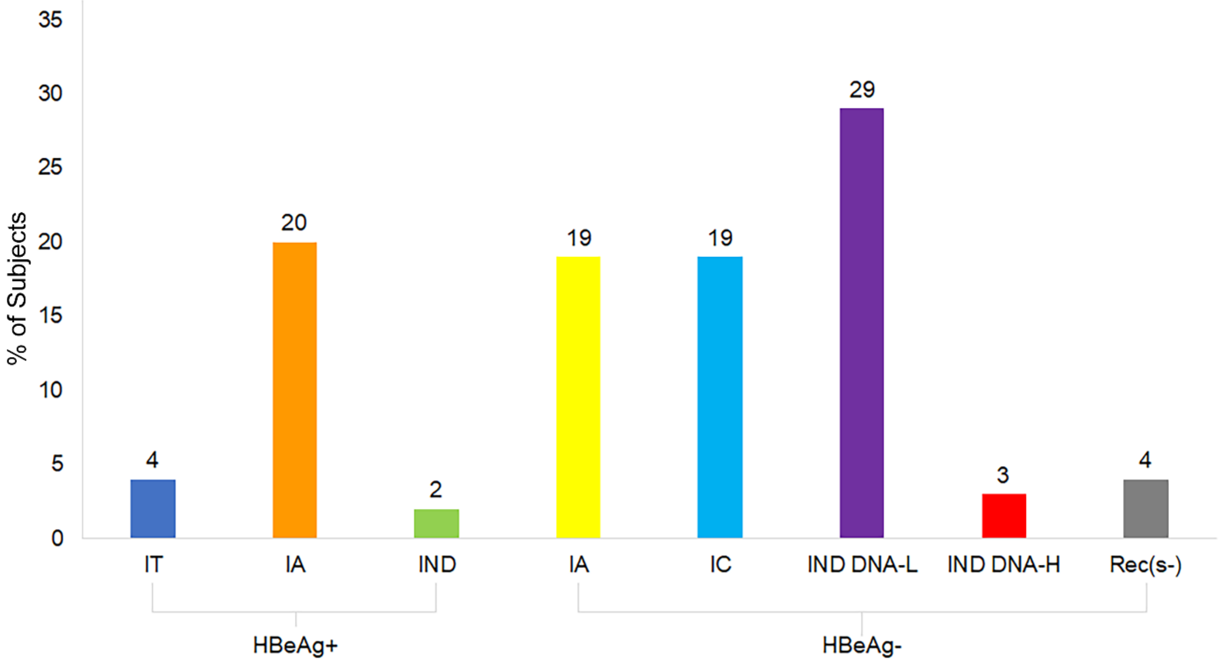
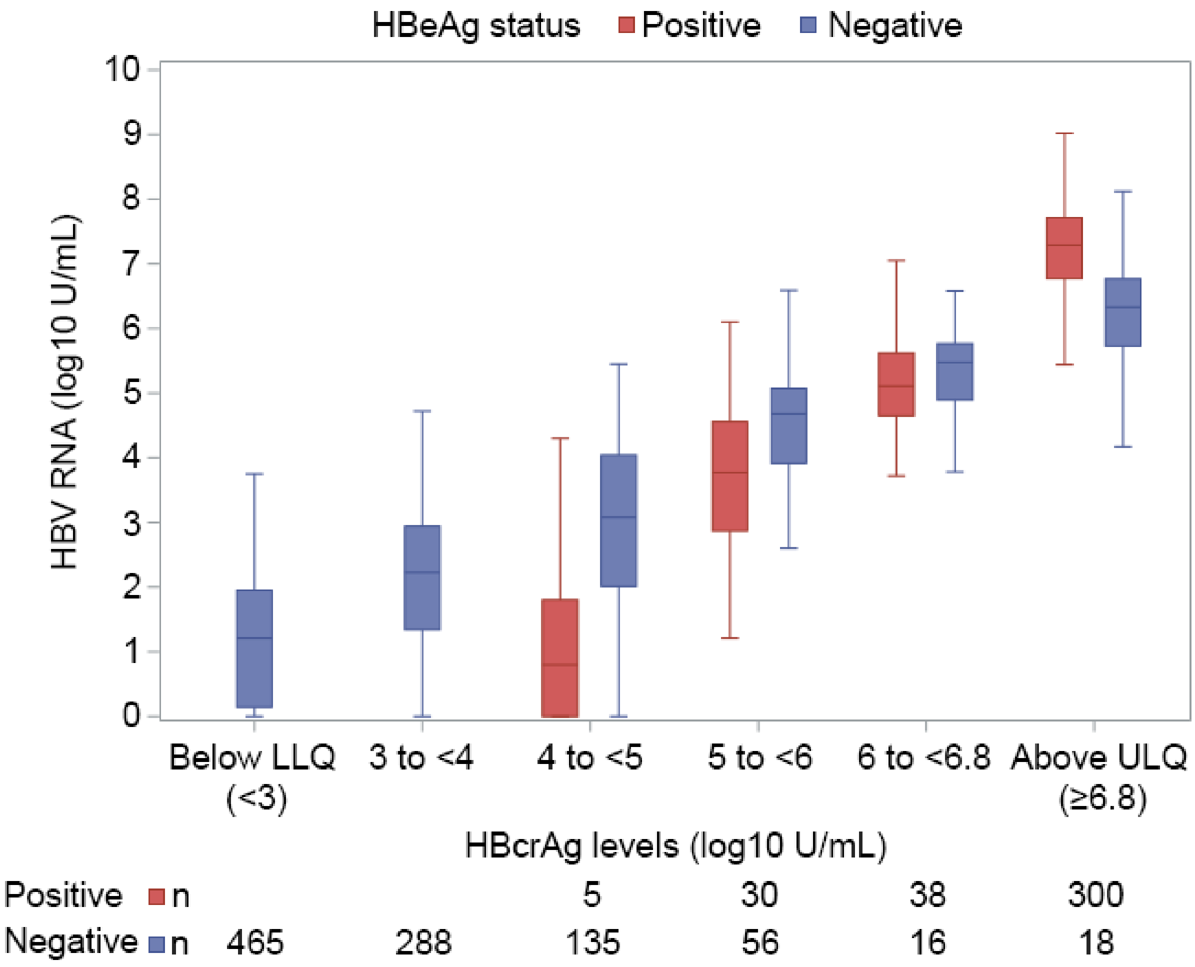


Figure S2: HBV Phase Allocation.



IT(e+) = immunetolerant, IA(e+) = HBeAg positive immuneactive, IND(e+) = indeterminant HBeAg positive, IA(e-) = HBeAg negative immuneactive , IC(e-) = inactive carrier, IND(e-)DNA-L = indeterminant HBeAg negative HBV DNA low, IND(e-)DNA-H = indeterminant HBeAg negative HBV DNA high, and Rec(s-) = Recovered HBsAg negative.

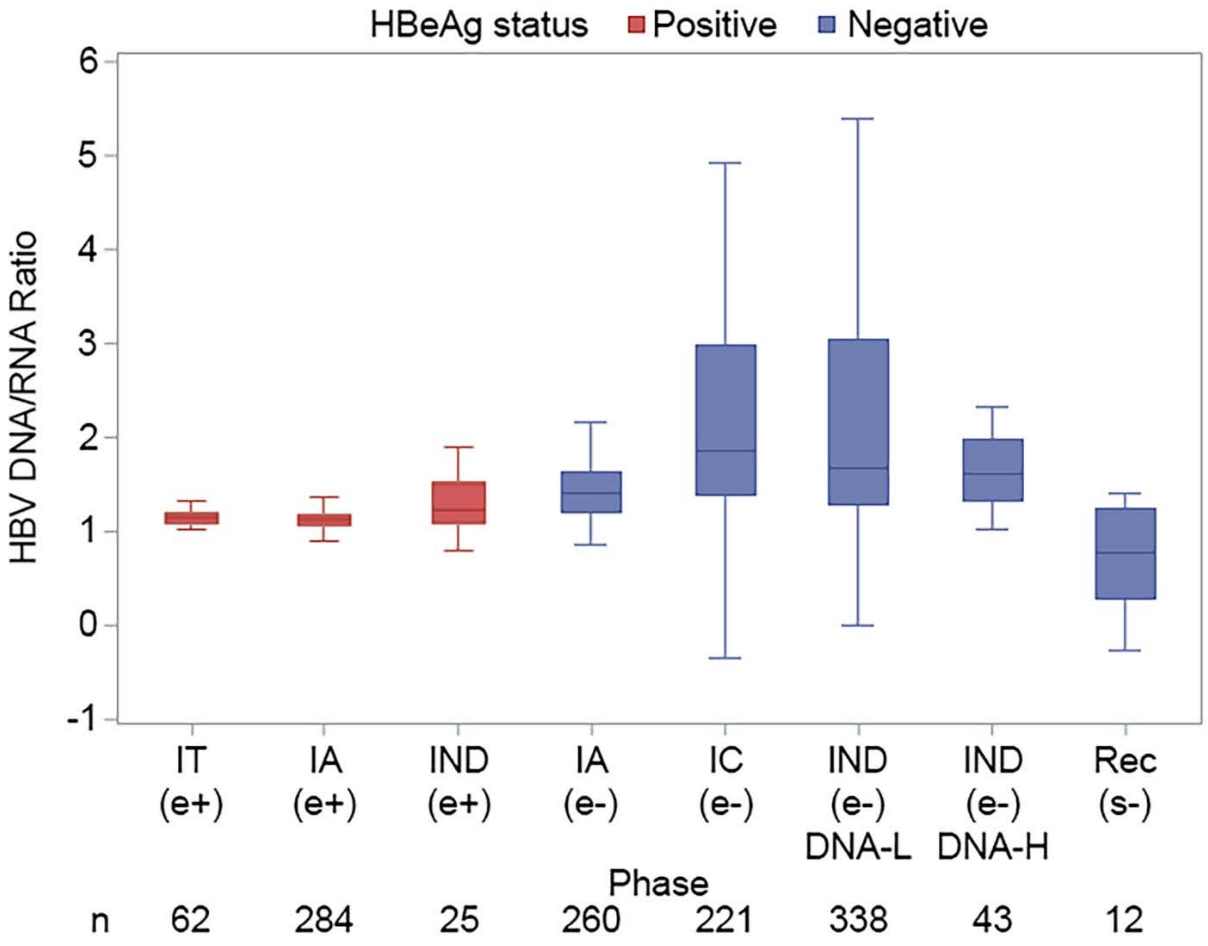
Figure S3: HBV RNA (log$_{10}$ U/mL) by HBcrAg (log$_{10}$ U/mL) categories, stratified by HBeAg status.



	Below LLQ (<3)	3 to <4	4 to <5	5 to <6	6 to <6.8	Above ULQ (≥6.8)
Positive 🟥 n			5	30	38	300
Negative 🟦 n	465	288	135	56	16	18

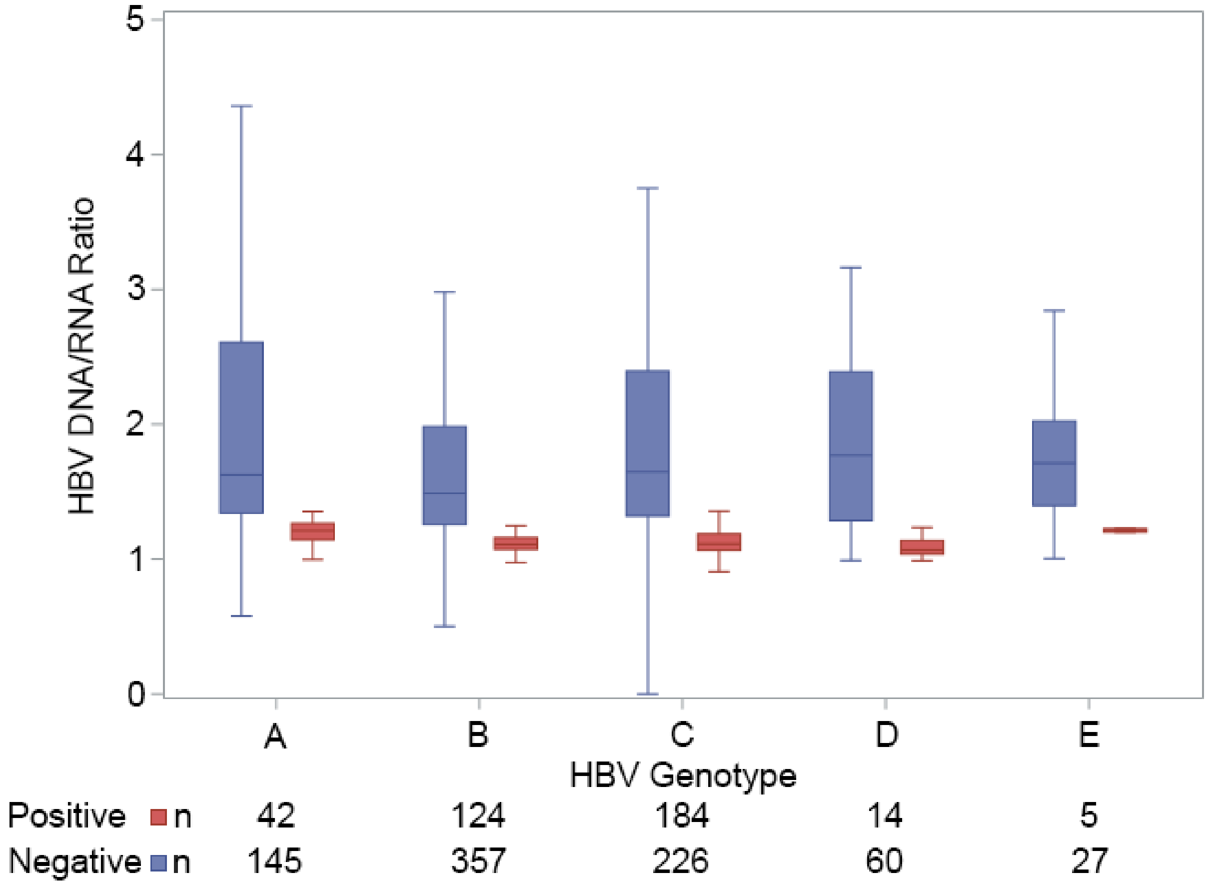
HBeAg positive participants depicted by red boxes and HBeAg negative participants by blue boxes. In box-whisker plots, upper and lower ends of the boxes = upper and lower quartlies, horizontal line = median, and upper and lower whiskers = highest and lowest observations.

Figure S4: HBV DNA/HBV RNA ratio by CHB Phase.



HBeAg positive participants depicted by red boxes and HBeAg negative participants by blue boxes. In box-whisker plots, upper and lower ends of the boxes = upper and lower quartlies, horizontal line = median, and upper and lower whiskers = highest and lowest observations. IT(e+) = immunetolerant, IA(e+) = HBeAg positive immuneactive, IA(e-) = HBeAg negative immuneactive, IC(e-) = inactive carrier, IND(e-)DNA-L = indeterminant HBeAg negative HBV DNA low, IND(e-)DNA-H = indeterminant HBeAg negative HBV DNA high, and Rec(s-) = Recovered HBsAg negative. n= numbers of participants in each CHB phase.

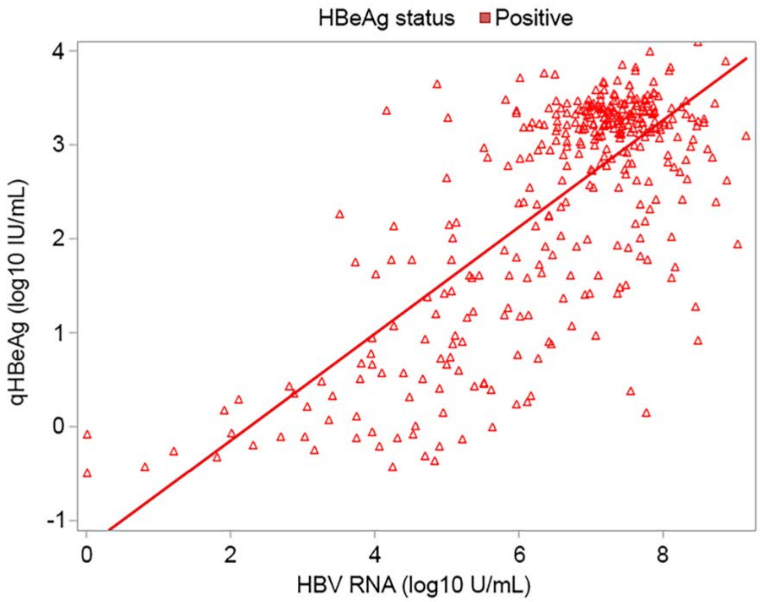
Figure S5: HBV DNA/HBV RNA ratio by genotype, stratified by HBeAg status.



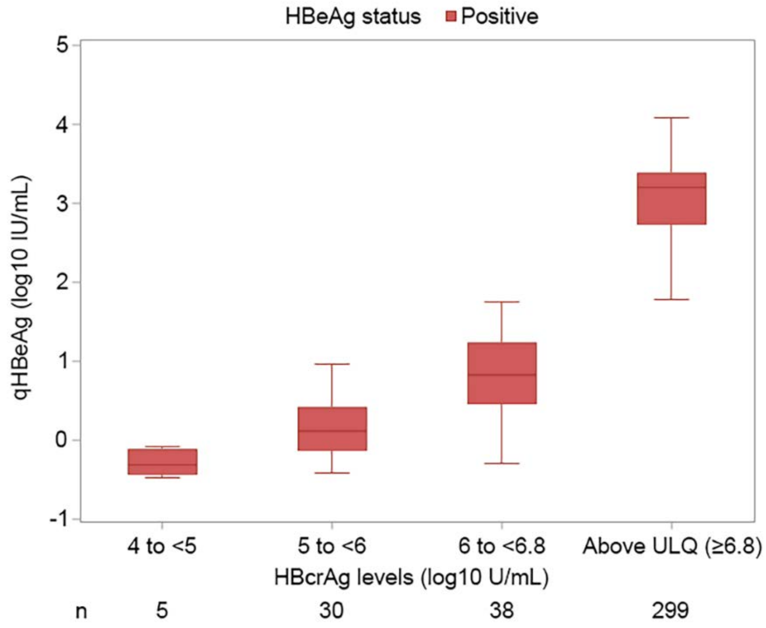
HBeAg positive participants depicted by red boxes and HBeAg negative participants by blue boxes. In box-whisker plots, upper and lower ends of the boxes = upper and lower quartlies, horizontal line = median, and upper and lower whiskers = highest and lowest observations.

Figure S6: qHBeAg (log10 IU/mL) by HBV RNA (log10 U/mL) and HBcrAg ($\log_{10}$ U/mL) categories, respectively, in HBeAg positive participants

A. qHBeAg by HBV RNA (participants depicted by red triangles)

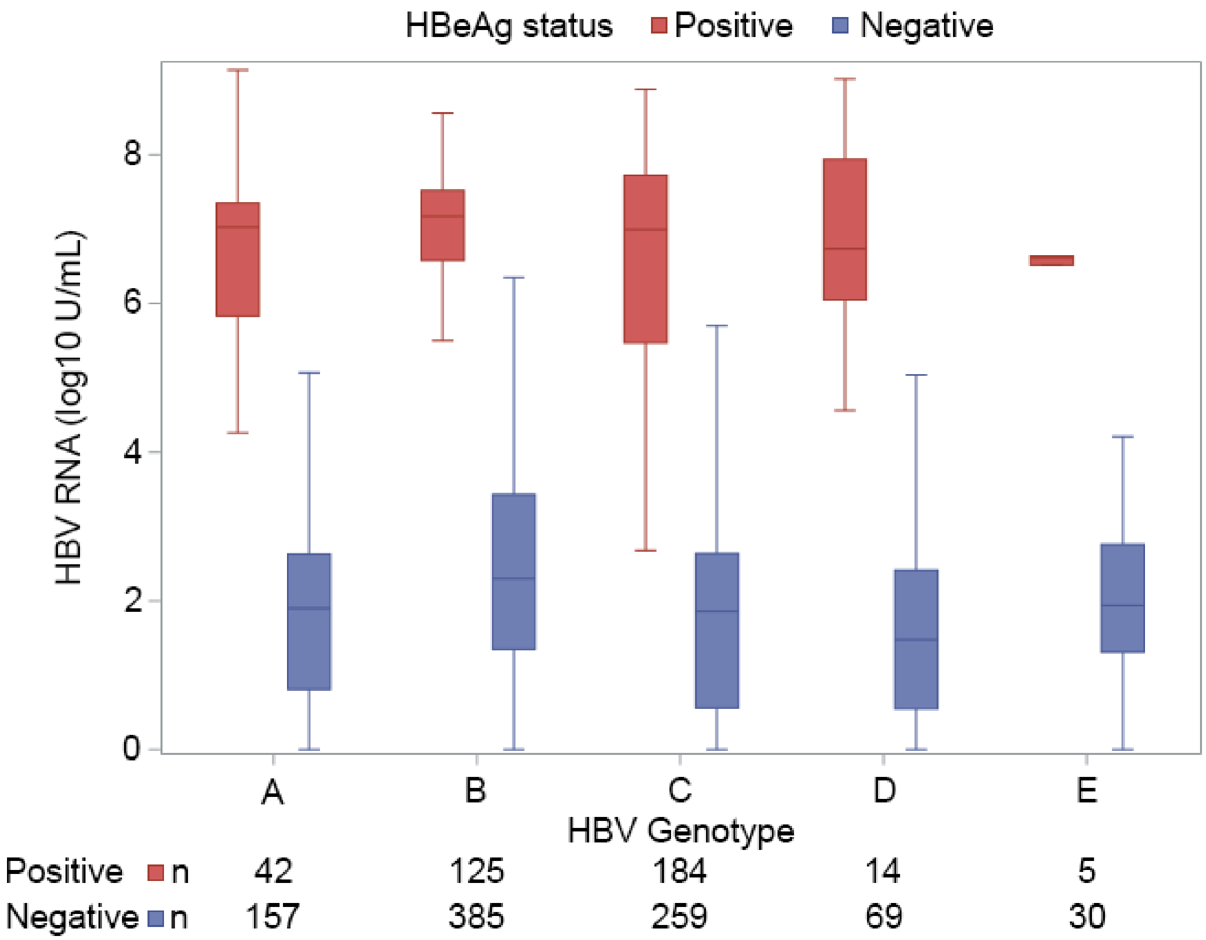


B. qHBeAg by HBcrAg categories



Upper and lower ends of the boxes = upper and lower quartlies, horizontal line = median, and upper and lower whiskers = highest and lowest observations.

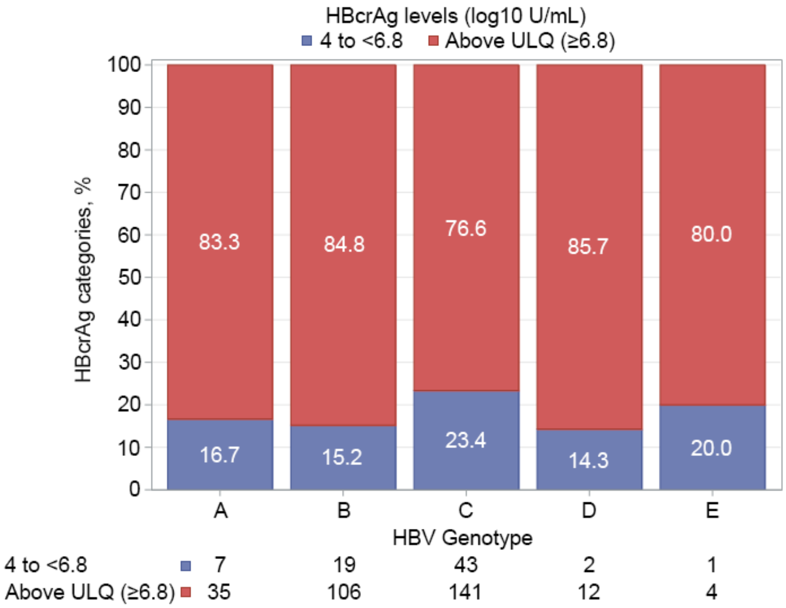
Figure S7: Distribution of HBV RNA ($\log_{10}$ U/mL) by genotype*, stratified by HBeAg status.



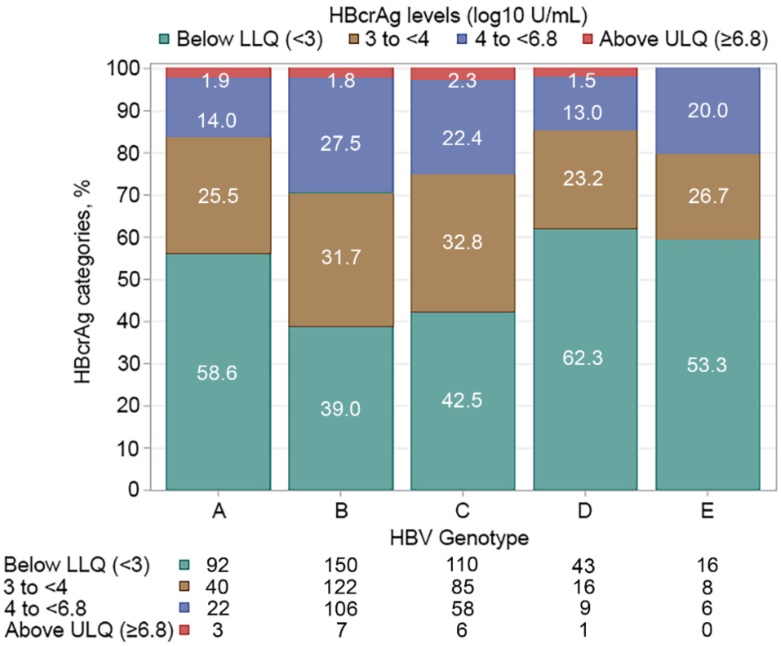
HBeAg positive participants depicted by red boxes and HBeAg negative participants by blue boxes. In box-whisker plots, upper and lower ends of the boxes = upper and lower quartlies, horizontal line = median, and upper and lower whiskers = highest and lowest observations.

Figure S8: Distribution of HBcrAg ($\log_{10}$ U/mL) categories by genotype, stratified by HBeAg status.

A. HBcrAg by HBV genotype in HBeAg positive participants



B. HBcrAg by HBV genotype in HBeAg negative participants

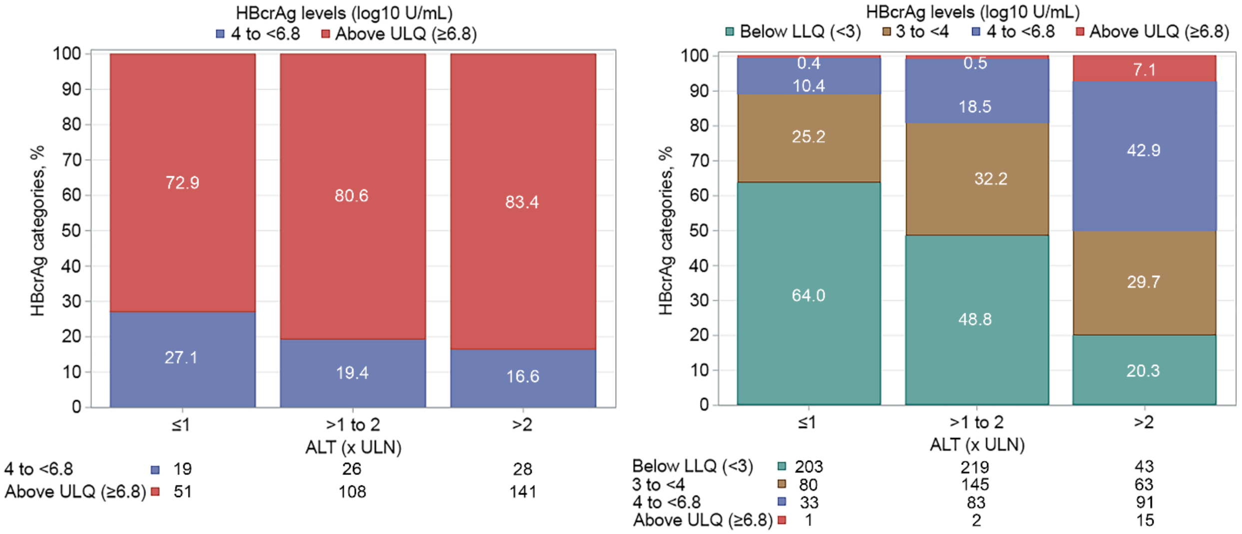


Green bars = HBcrAg below lower limit of detection, brown bars = HBcrAg 3-<4 U/L, blue bars = HBcrAg 4-<6.8 U/L and red bars = HBcrAg≥6.8 U/L.

Figure S9: HBcrAg (log10 IU/mL) categories by ALT (ULN), APRI and FIB-4 categories, stratified by HBeAg status.

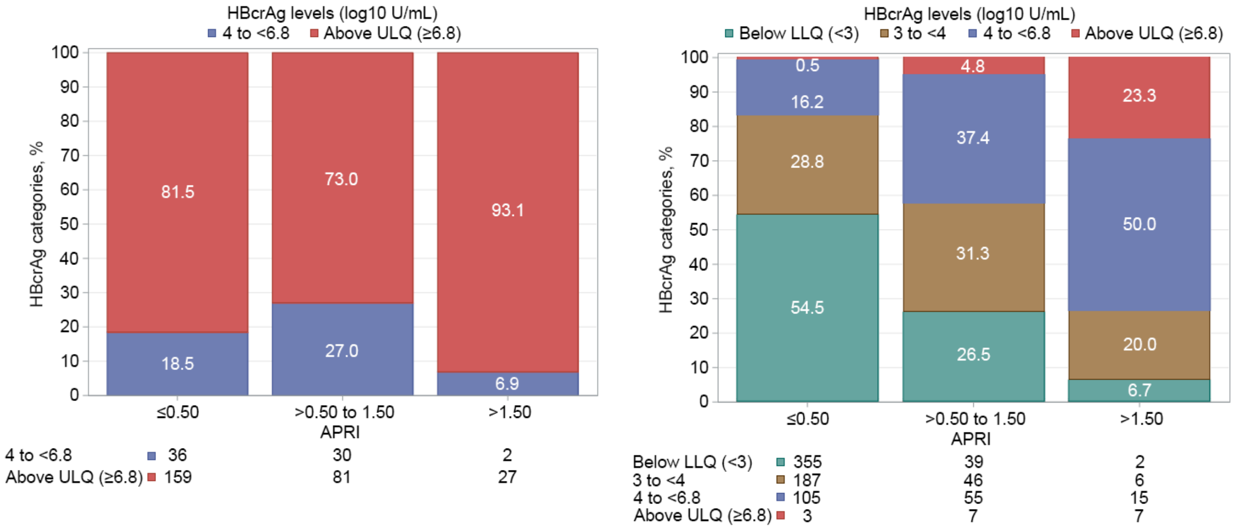
A. HBcrAg by ALT among HBeAg+

B. HBcrAg by ALT among HBeAg-

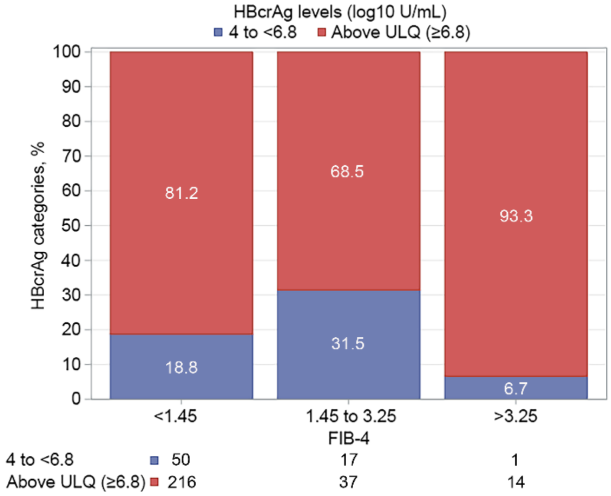


C. HBcrAg by APRI among HBeAg+

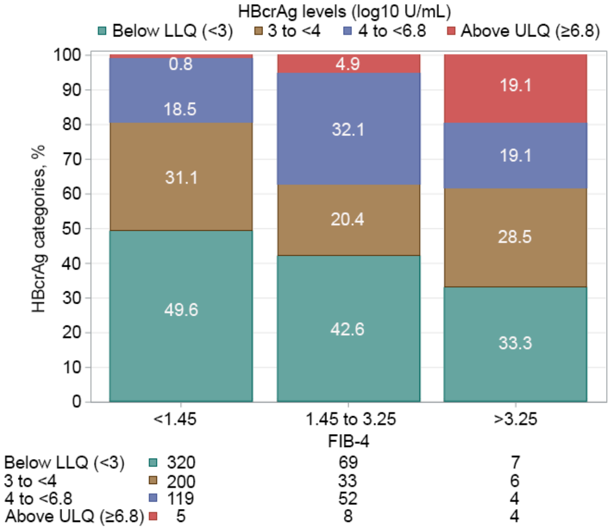
D. HBcrAg by APRI among HBeAg-



E. HBcrAg by FIB-4 among HBeAg+



F. HBcrAg by FIB-4 among HBeAg-

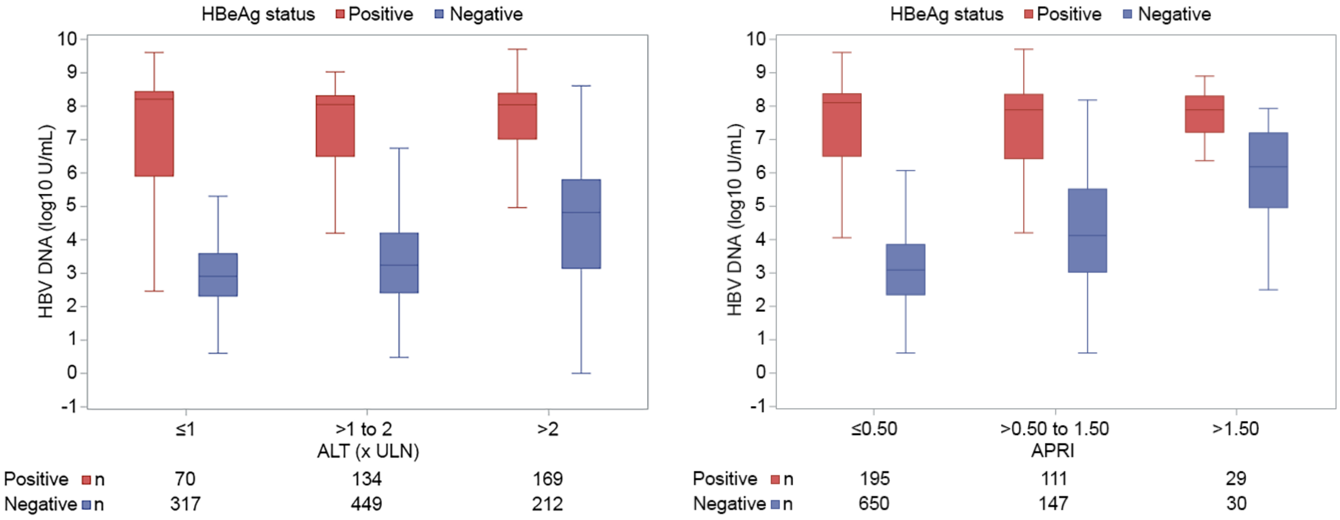


Green bars = HBcrAg below lower limit of detection, brown bars = HBcrAg 3-<4 U/L, blue bars = HBcrAg 4-<6.8 U/L and red bars = HBcrAg≥6.8 U/L.

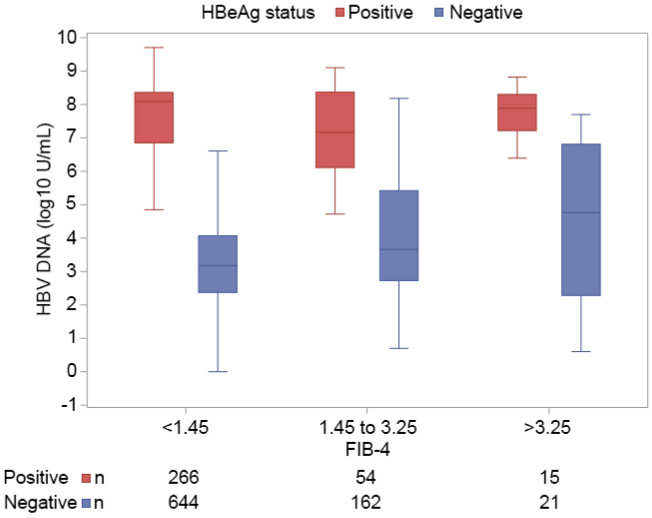
Figure S10: HBV DNA (log10 U/mL) by ALT (ULN), APRI and FIB-4 categories

A. HBV DNA by ALT

B. HBV DNA by APRI

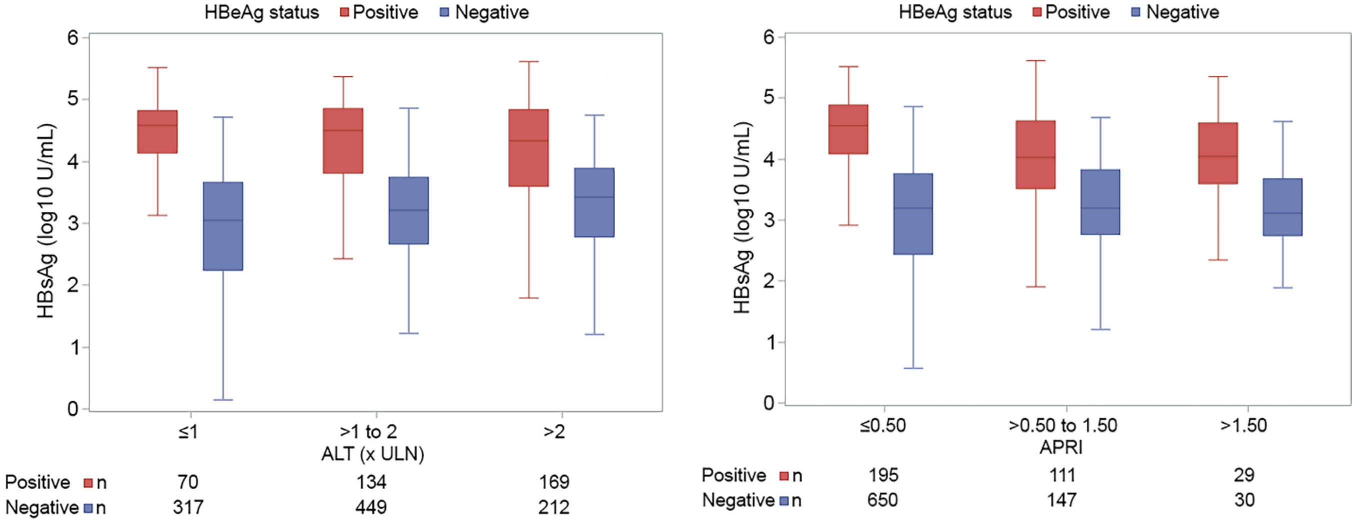


C. HBV DNA by FIB-4



A, B and C, HBeAg positive particpants represented by red boxes and HBeAg negative participants by blue boxes. In box-whisker plots, upper and lower ends of the boxes = upper and lower quartlies, horizontal line = median, and upper and lower whiskers = highest and lowest observations.

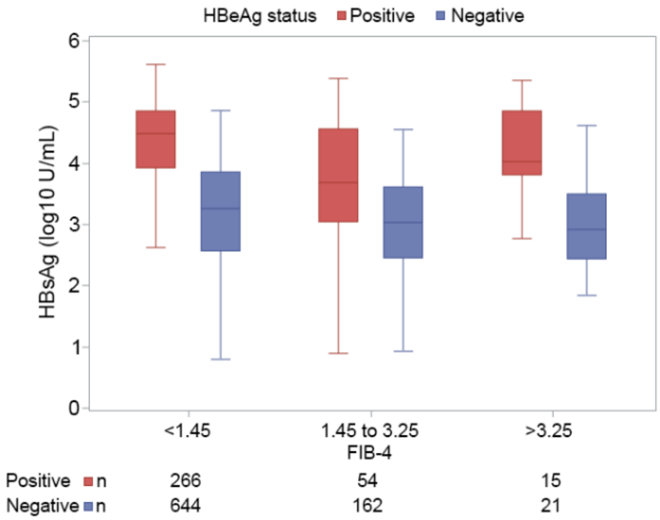
Figure S11: HBsAg (log10 U/mL) by ALT (ULN), APRI and FIB-4 categories



A. HBsAg by ALT

B. HBsAg by APRI

C. HBsAg by FIB-4



A, B and C, HBeAg positive particpants represented by red boxes and HBeAg negative participants by blue boxes. In box-whisker plots, upper and lower ends of the boxes = upper and lower quartlies, horizontal line = median, and upper and lower whiskers = highest and lowest observations.

Table S1. Associations between HBV RNA, HBcrAg, HBV DNA and qHBsAg, respectively, with ALT, APRI and FIB-4 categories*, respectively, among HBeAg positive particiapnts.

	ALT categories		**APRI categories**		**FIB-4 categories**	
	Unadjusted models					
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
HBV RNA-per log10 U/mL		0.007		0.25		0.56
2 vs 1	1.07 (0.90-1.27)		1.04 (0.90-1.21)		0.90 (0.76-1.07)	
3 vs 1	1.26 (1.05-1.50)		1.24 (0.92-1.68)		1.17 (0.78-1.74)	
HBcrAg (≥6.8 vs 4-<6.8 log10 U/mL)		0.08		0.67		0.27
2 vs 1	1.55 (0.79-3.05)		0.61 (0.35-1.06)		0.50 (0.26-0.97)	
3 vs 1	1.88 (0.97-3.65)		3.06 (0.70-13.44)		3.24 (0.42-25.22)	
HBV DNA-per log10 U/mL		0.056		0.71		0.59
2 vs 1	1.02 (0.85-1.23)		0.97 (0.83-1.13)		0.90 (0.75-1.08)	
3 vs 1	1.17 (0.97-1.41)		1.20 (0.88-1.65)		1.19 (0.78-1.83)	
qHBsAg-per log10 U/mL		0.25		0.009		<0.001
2 vs 1	0.92 (0.64-1.31)		0.69 (0.52-0.91)		0.53 (0.39-0.72)	
3 vs 1	0.83 (0.59-1.17)		0.72 (0.47-1.11)		0.88 (0.47-1.65)	
	Multivariable models**					
	adjOR (95% CI)	**p-value**	**adjOR (95% CI)**	**p-value**	**adjOR (95% CI)**	**p-value**
HBV RNA-per log10 U/mL		<.001		0.045		0.29
2 vs 1	1.09 (0.90-1.32)		1.11 (0.94-1.31)		0.91 (0.76-1.10)	
3 vs 1	1.39 (1.12-1.71)		1.41 (1.01-1.95)		1.27 (0.84-1.93)	
HBcrAg (≥6.8 vs 4-<6.8 log10 U/mL)		0.01		0.66		0.14
2 vs 1	1.69 (0.79-3.60)		0.77 (0.42-1.40)		0.58 (0.29-1.15)	
3 vs 1	2.60 (1.21-5.59)		3.76 (0.83-17.01)		3.25 (0.41-25.90)	
HBV DNA-per log10 U/mL		0.008		0.34		0.38
2 vs 1	1.04 (0.85-1.28)		1.02 (0.86-1.20)		0.91 (0.75-1.10)	
3 vs 1	1.27 (1.02-1.57)		1.28 (0.92-1.79)		1.21 (0.78-1.87)	
qHBsAg-per log10 U/mL		0.71		0.10		<0.001

2 vs 1	0.95 (0.63-1.44)		0.77 (0.57-1.04)		0.52 (0.37-0.72)	
3 vs 1	0.91 (0.61-1.36)		0.79 (0.51-1.24)		0.82 (0.44-1.56)	

*ALT (ULN) categories: 1: ≤1.0-2: >1.0-2.0-3: >2.0; APRI categories: 1: ≤0.5-2: 0.5-2.0-3: >2.0; FIB-4 categories: 1: <1.45-2: 1.45-3.25-2: >3.25.

**Adjusted for age (years) and body mass index (kg/m^2).

Table S2. Associations between HBV RNA, HBcrAg, HBV DNA and qHBsAg, respectively, with ALT, APRI and FIB-4 categories*, respectively, among HBeAg negative participants.

	ALT categories		**APRI categories**		**FIB-4 categories**	
	Unadjusted models					
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
HBV RNA-per log10 U/mL		<.0001		<.0001		<.0001
2 vs 1	1.30 (1.17-1.46)		1.64 (1.45-1.86)		1.32 (1.19-1.47)	
3 vs 1	2.17 (1.89-2.49)		2.97 (2.30-3.85)		1.69 (1.33-2.16)	
HBcrAg 3-<4 vs <3 log10 U/mL)		<.0001		<.0001		<.0001
2 vs 1	1.68 (1.20-2.34)		2.24 (1.41-3.55)		0.77 (0.49-1.20)	
3 vs 1	3.72 (2.33-5.93)		5.70 (1.14-28.49)		1.37 (0.45-4.14)	
HBcrAg 4-<6.8 vs <3 log10 U/mL)						
2 vs 1	2.33 (1.49-3.64)		4.77 (3.00-7.59)		2.03 (1.34-3.08)	
3 vs 1	13.02 (7.77-21.82)		25.36 (5.71-112.67)		1.54 (0.44-5.34)	
HBcrAg ≥6.8 vs <3 log10 U/mL)						
2 vs 1	1.86 (0.17-20.61)		21.22 (5.28-85.39)		7.42 (2.36-23.37)	
3 vs 1	70.81 (9.11-550.56)		-		36.57 (8.05-166.06)	
HBV DNA-per log10 U/mL		<.0001		<.0001		<.0001
2 vs 1	1.27 (1.14-1.43)		1.71 (1.49-1.95)		1.38 (1.23-1.54)	
3 vs 1	2.19 (1.90-2.52)		3.32 (2.50-4.43)		1.62 (1.24-2.11)	
qHBsAg-per log10 U/mL		<.0001		0.08		0.01
2 vs 1	1.26 (1.08-1.46)		1.16 (0.95-1.41)		0.81 (0.68-0.96)	
3 vs 1	1.46 (1.20-1.77)		1.25 (0.83-1.89)		0.84 (0.55-1.29)	
	Multivariable models**					
	adjOR (95% CI)	**p-value**	**adjOR (95% CI)**	**p-value**	**adjOR (95% CI)**	**p-value**
HBV RNA-per log10 U/mL		<.0001		<.0001		<.0001

2 vs 1	1.35 (1.19-1.52)		1.64 (1.45-1.86)		1.33 (1.19-1.48)	
3 vs 1	2.28 (1.97-2.64)		2.87 (2.21-3.73)		1.55 (1.20-2.00)	
HBcrAg 3-<4 vs <3 log10 U/mL)		<.0001		<.0001		<.0001
2 vs 1	4.63 (2.79-7.70)		8.20 (1.60-42.11)		1.38 (0.45-4.17)	
3 vs 1	1.90 (1.32-2.73)		2.96 (1.81-4.84)		0.78 (0.49-1.24)	
HBcrAg 4-<6.8 vs <3 log10 U/mL)						
2 vs 1	3.01 (1.84-4.94)		6.26 (3.80-10.31)		2.02 (1.32-3.10)	
3 vs 1	19.13 (10.75-34.03)		33.93 (7.31-157.48)		1.14 (0.29-4.51)	
HBcrAg ≥6.8 vs <3 log10 U/mL)						
2 vs 1	1.98 (0.18-22.14)		20.89 (4.91-88.88)		8.81 (2.58-30.10)	
3 vs 1	69.27 (8.65-555.02)		-		31.60 (5.92-168.68)	
HBV DNA-per log10 U/mL		<.0001		<.0001		<.0001
2 vs 1	1.34 (1.19-1.52)		1.72 (1.50-1.97)		1.37 (1.22-1.55)	
3 vs 1	2.40 (2.05-2.80)		3.21 (2.39-4.32)		1.42 (1.06-1.89)	
qHBsAg-per log10 U/mL		<.0001		0.01		0.008
2 vs 1	1.29 (1.09-1.52)		1.30 (1.04-1.63)		0.80 (0.67-0.95)	
3 vs 1	1.52 (1.22-1.88)		1.41 (0.88-2.27)		0.75 (0.49-1.15)	

*ALT (ULN) categOR (95% CI)ies: 1: ≤1.0-2: >1.0-2.0-3: >2.0; APRI categOR (95% CI)ies: 1: ≤0.5-2: 0.5-2.0-3: >2.0; FIB-4 categOR (95% CI)ies: 1: <1.45-2: 1.45-3.25-2: >3.25.

**Adjusted for age (years) and body mass index (kg/m^2).