A Higher Proportion of Unglycosylated HBsAg is Predictive of Faster HBsAg Decline Kinetics In Patients with Chronic Hepatitis B

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1313

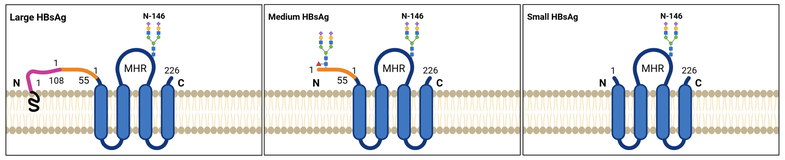
Plain Language Summary

• This study looked at the relationship between proportion of unglycosylated Hepatitis B surface antigen (HBsAg) level and HBsAg decline in patients treated by Nucleos(t)ide analogues (NAs)
•We found that a higher proportion of unglycosylated HBsAg (pS) is associated with faster HBsAg decline kinetics among patients with Chronic Hepatitis B (CHB) treated by NAs
•Patients who achieved HBsAg loss also had a higher proportion of unglycosylated HBsAg than those who did not achieve HBsAg loss
• This suggests that a higher proportion of unglycosylated HBsAg may serve as a predictive biomarker for faster HBsAg decline

Introduction

- A functional cure for Chronic Hepatitis B (CHB), defined as sustained loss of HBsAg and sustained suppression of HBV DNA, is a key treatment goal[1]
- There is a lack of robust biomarkers predicting early-stage Hepatitis B surface antigen (HBsAg) decline in patients with CHB[1]
- Small HBsAg can be glycosylated via an N-linked glycosylation site at amino acid 146 (N146), which is important for immune recognition, protein folding, and viral secretion[2,3]
- In most CHB patients, the ratio of serum unglycosylated (pS) to glycosylated (GpS) HBsAg (pS/GpS) is approximately 1:1 since N146 is not fully utilized[2,3]
- However, the relationship between HBsAg glycosylation level and HBsAg decline is poorly understood in CHB patients

Glycosylation sites on HBsAg



All forms of HBsAg (large HBsAg, medium HBsAg and small HBsAg) have glycosylation site N146. MHR: Major hydrophilic region
Figures were generated using BioRender.

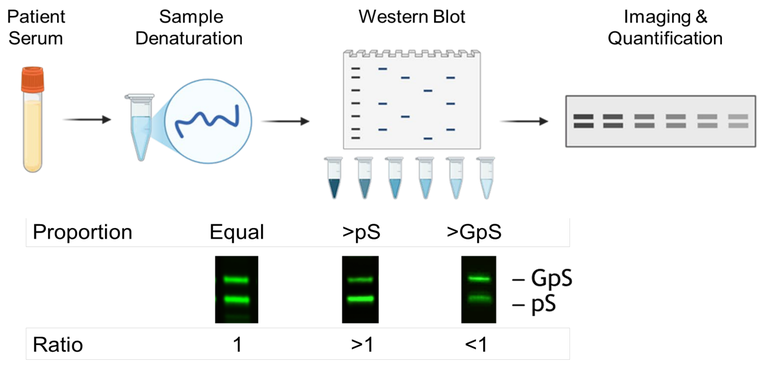
Objective

- To investigate the association between proportion of unglycosylated HBsAg and HBsAg decline in CHB patients, to understand the HBsAg loss mechanism related to glycosylation level of HBsAg

Methods

- Baseline (BL) serum samples were randomly selected from CHB patients in two similarly designed phase 3 studies of tenofovir alafenamide and/or tenofovir disoproxil fumarate: GS-US-320-0108 (HBeAg-) and GS-US-320-0110 (HBeAg+)[4]
- Western blot analysis was used to quantify pS and GpS band intensities, from which pS/GpS ratios were calculated
- Quantitative HBsAg levels were measured every 12 weeks through week 144, and every 24 weeks until week 384. HBsAg decline was analyzed from BL to week 384. BL sequences were analyzed to detect mutations at N146
- All correlation analyses used Pearson's method unless otherwise noted

Using western blot to detect and quantify HBsAg from patient samples



GpS: glycosylated HBsAg; pS: unglycosylated HBsAg

References: **1**. Charre C, et al. Antiviral Research. 2019;169;104553. **2**. Julithe R, etal. J Virol. 2014;88(16):9049-9059. **3**. Dobrica MO, et al. Cells. 2020;9(6):1404. 4. Buti M, et al. Aliment Pharmacol Ther. 2024;60(11-12):1573-1586.

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Disclosures: **CL, AL, NP, SM, JL, CR, CM, RM, FA, HM, and EM** are employees of Gilead Sciences, Inc., and may own stock in Gilead Sciences, Inc.
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Results

Patient characteristics

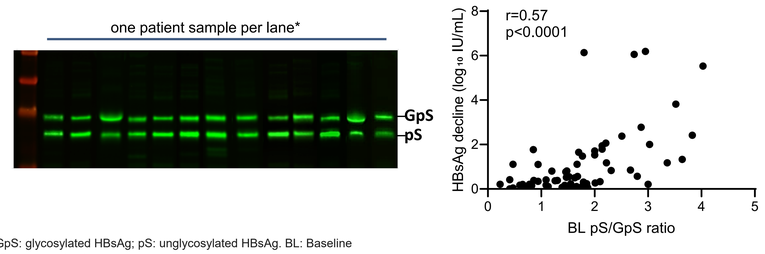
- A total of 66 patients (48 HBeAg+ and 18 HBeAg-), with HBV genotypes (GTs): GTA (N = 11, 16.7%), GTB (N = 24, 36.4%), GTC (N = 30, 45.5%), and GTF (N = 1, 1.5%) were analyzed
- The median levels of HBV DNA were 4.72 log10 IU/mL in HBeAg- patients and 8.05 log10 IU/mL in HBeAg+ patients, respectively
- Median HBsAg levels were 3.24 log10 IU/mL in HBeAg- patients and 4.16 log10 IU/mL in HBeAg+ patients

Baseline characteristics of N=66 patients included in the analysis

	HBeAg- N=18	HBeAg+ N=48	Total N=66
HBV DNA (log10 IU/mL)	4.72 (4.15, 6.52)	8.05 (7.45, 8.56)	**7.50 (6.40, 8.50)**
ALT (U/L)	64 (41, 95)	87 (66, 141)	**82 (60, 132)**
HBsAg (log10 IU/mL)	3.24 (2.86, 3.34)	4.16 (3.78, 4.65)	**3.84 (3.36, 4.45)**
Male, n (%)	11 (61.1)	31 (64.6)	**41 (62.1)**
HBV genotype, n (%)			
GTA	1 (5.6)	10 (20.8)	**11 (16.7)**
GTB	7 (38.9)	17 (35.4)	**24 (36.4)**
GTC	10 (55.6)	20 (41.7)	**30 (45.5)**
GTF	0 (0)	1 (2.1)	**1 (1.5)**

ALT, alanine aminotransferase; GT, genotype; HBeAg, Hepatitis B e Antigen; HBsAg, Hepatitis B surface Antigen
Median (Q1, Q3) was shown for HBV DNA, ALT, and HBsAg levels

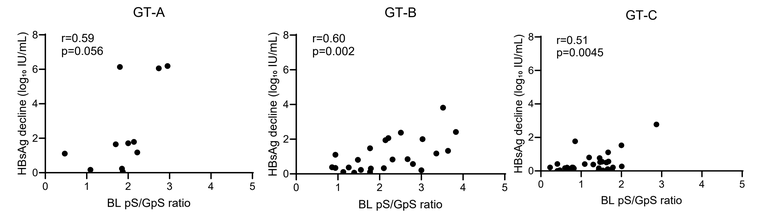
Relationship between BL pS/GpS ratio and HBsAg decline



GpS: glycosylated HBsAg; pS: unglycosylated HBsAg. BL: Baseline
*Representative image

- BL ratio (pS/GpS) ranged from 0.23 to 4.03. HBsAg decline from BL to week 48 ranged from 0 to 6.19 log
- Higher BL pS/GpS ratio is positively associated with greater HBsAg decline from BL to week 48 (r=0.57, p<0.001) in all patients
- Similar correlations persisted from weeks 96 to 196 (r>0.5, p<0.001) (data not shown)

Baseline pS/GpS ratio was associated with HBsAg decline from BL to week 48 in patients with different genotypes



BL: Baseline; GT: Genotype.

- Week 48 HBsAg decline and baseline pS/GpS ratio in patients with different genotypes (A, B, and C) is positively correlated: GTA (r=0.59, p=0.056), GTB (r=0.60, p=0.002), and GTC (r=0.51, p=0.0045)

Higher BL pS/GpS ratio was associated with faster HBsAg loss

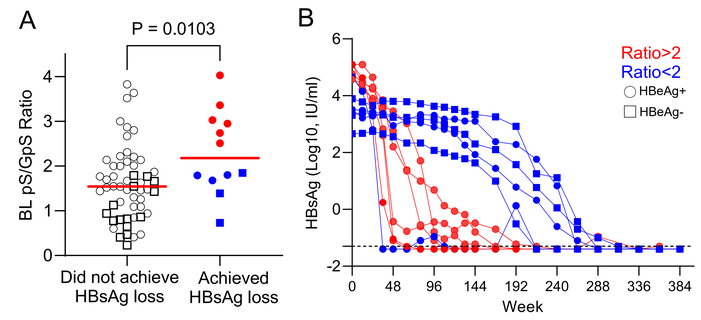
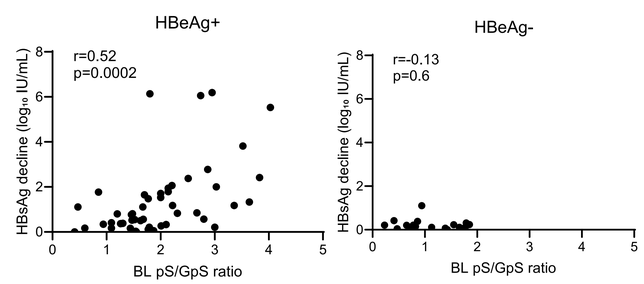


Figure A. BL pS/GpS ratios by HBsAg loss status. Figure B. HBsAg levels over time by BL pS/GpS ratio.
GpS: glycosylated HBsAg; pS: unglycosylated HBsAg. BL: Baseline
Median value was shown in red bar.

- Patients who achieved HBsAg loss (N=12) had significantly higher baseline pS/GpS ratios than those who did not achieve HBsAg loss (N=54) (p=0.0103, Student t test) (Figure A)
- In HBsAg loss patients, a higher ratio (ratio>2) was associated with faster HBsAg loss, while a lower ratio (ratio<2) was associated with slower HBsAg loss (Figure B)

Baseline pS/GpS ratio was associated with HBsAg decline from BL to week 48 in HBeAg+ CHBs



GpS: glycosylated HBsAg; pS: unglycosylated HBsAg
BL: Baseline

- HBsAg decline from BL to week 48 was significantly associated with the BL ratio (pS/GpS) in HBeAg+ patients (r=0.52, p<0.0002) but not in HBeAg- patients (r=-0.13, p=0.6)

Conclusions

- The BL pS/GpS ratio is strongly correlated with HBsAg decline from baseline to week 48 in HBeAg+ CHB patients in these studies (GS-US-320-0108 and GS-US-320-0110), suggesting its potential as a predictive biomarker of faster HBsAg decline in HBeAg+ patients with CHB
- Patients with HBV GTs A, B, and C showed a positive correlation between BL pS/GpS ratio and HBsAg decline from baseline to week 48
- Patients who achieved HBsAg loss had significantly higher baseline pS/GpS ratios than those who did not achieve HBsAg loss
- In HBsAg loss patients, a higher ratio was associated with faster HBsAg loss kinetics, while a lower ratio was associated with slower kinetics of HBsAg loss
- No mutations at N146 were found in any samples at BL
- Small sample size is one of the limitations of this study
- Further investigations are needed to understand the underlying mechanism of HBsAg loss related to glycosylation level of HBsAg

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