Circulating HBV RNA correlates with intrahepatic covalently closed circular DNA (cccDNA) levels and activity in untreated chronic hepatitis B (CHB) patients

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INTRODUCTION

Complete recovery from chronic hepatitis B (CHB) is seldom achieved, due to the persistence in the liver of the viral covalently closed circular DNA (cccDNA), which remains unaffected by current antiviral therapies.

Routine circulating biomarkers used for clinical monitoring of patients, serum HBV-DNA and HBsAg, do not accurately reflect intrahepatic cccDNA pool and transcriptional activity, therefore new biomarkers better reflecting viral activity in the liver are sorely needed.

AIM

To provide a comprehensive **analysis of Hepatitis B virus (HBV) RNA in blood circulation (cirB-RNA) in the different chronic hepatitis B (CHB) phases and its correlation with intrahepatic viral markers and HBcrAg,** the other emerging biomarker of cccDNA transcription.

MATERIAL & METHODS

122 untreated CHB patients with **paired liver biopsy and serum sample**, 32 HBeAg(+) chronic hepatitis (CH), 29 HBeAg(-) chronic infection (CI) and 61 HBeAg(-) CH, were analyzed for **serum HBV DNA**, quantitative **(q)HBsAg**, **HBcrAg** and alanine aminotransferase (**ALT**) **levels**. Liver **cccDNA** and **3.5Kb RNA** were assessed by qPCR and RT-qPCR, respectively, and **cccDNA transcriptional activity** was calculated as 3.5Kb RNA/cccDNA ratio. Liver histology scores were also available.

Circulating HBV RNA was quantified by real-time PCR using the **Roche HBV RNA investigational assay (IA) for use on the cobas® 6800/8800 Systems** (Roche Diagnostics, Pleasanton, CA, USA). The HBV RNA assay is a quantitative nucleic acid test (**LLOQ 10 cp/ml**; linearity range 10 to 10^9cp/ml on armored RNA template) to enable the detection and quantification of HBV RNA in EDTA plasma or serum of HBV-infected patients. All tests were performed by trained operators in accordance with the manufacturers' specifications. Runs were considered valid if internal controls were valid and no protocol deviations or incidents occurred that might affect the validity of the data. If a run was considered invalid, all samples included in that run were retested wherever possible.

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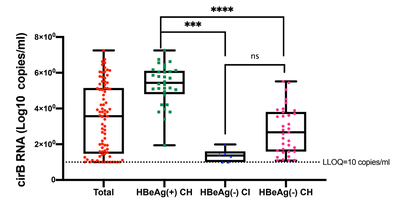
RESULTS

Table 1. Patients' clinical characteristics

	Total cohort (n=122)	HBeAg(+) (n=32)	HBeAg(-) patients (n=90)		*p-value*[2]
		cirB RNA(+) (n=32)	cirB RNA(+) (n=51)	cirB RNA(–) (n=39)	
Age[1] (years)	40.2 (28.6 – 50.1)	32.4 (24-43.8)	44.2 (30.4 – 52.5)	43.3 (33.8 -54.4)	ns[3]
Sex (M/F)	86/36 (70/30%)	25/7 (80/20%)	36/15 (70/30%)	25/14 (64/36%)	ns[4]
Origin					
Caucasian	42 (34.5 %)	12 (37.5 %)	18 (35 %)	12 (31 %)	-
Middle East	13 (11 %)	3 (9 %)	6 (12 %)	4 (10 %)	
Asian	26 (21 %)	10 (32 %)	9 (18 %)	7 (18 %)	
North Africa	16 (13 %)	4 (12.5 %)	7 (14 %)	5 (13 %)	
Sub-s. Africa	25 (20.5 %)	3 (9 %)	11 (21 %)	11 (28 %)	
Viral genotype					
A	20 (16 %)	9 (28 %)	5 (10 %)	6 (15.4 %)	ns[4]
B	3 (2.5 %)	1 (3 %)	0 (0 %)	2 (5 %)	
C	11 (9 %)	7 (23 %)	3 (6 %)	1 (2.5 %)	
D	48 (39.5 %)	9 (28 %)	29 (57 %)	10 (25.6 %)	
E	14 (11.5 %)	1 (3 %)	6 (12 %)	7 (18 %)	
F	3 (2.5 %)	2 (6 %)	0 (0 %)	1 (2.5 %)	
ND	23 (9 %)	3 (9 %)	8 (15 %)	12 (31 %)	
Viral load[1] (logIU/mL)	4.4 (2.9 – 7.2)	8 (7.4-8.6)	4.5 (3.5-6.1)	3 (2.4-5.3)	<0.0001[3]
ALT[1] (IU)	52.5 (34 – 79.2)	96.5 (67-203.3)	50 (35.5 – 65.5)	39 (26.5–65)	0.007[3]
qHBsAg[1] (logIU/ml)	3.9 (3.4 – 4.3)	4.6 (3.9-5.2)	3.7 (3.1-4)	4 (3.3-4.5)	ns[3]
HBcrAg (+/-)	89/33 (73/27%)	32/0 (100/0%)	40/11 (76/24%)	17/22 (43.5/56.5%)	0.002[4]
HBcrAg[1] (logIU/ml)	4.8 (3.8 – 7.5)	8 (7.3-8.4)	4.1 (3.7 – 5.1)	3.5 (3.1 – 4)	0.004[3]
cirB RNA[1] (log_{10} copies/ml)	3.9 (2-5.4)	5.4 (4.8-6.11)	2.3 (1.5-3.7)	-	-
Fibrosis (≤1/>2)	67/55 (55/44%)	15/17 (47/53%)	23/28 (45/55%)	29/10 (74.4/25.6%)	0.009[4]

[1]Data are expressed as median (1st-3rd quartile); [2]comparison between HBeAg(-) cirB RNA(+) vs cirB RNA(-); [3]Mann-Whitney test, α threshold=0.5; [4]Fischer exact test or χ^2 test

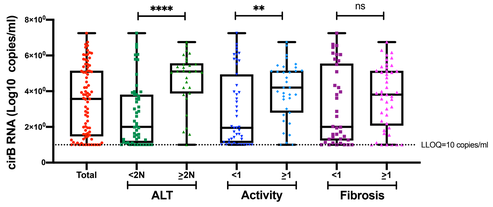
Figure 1a. cirB RNA distribution across CHB phases…



	Total	HBeAg(+) CH	HBeAg(-) CI	HBeAg(-) CH
Positive/Total	83/122	32/32	6/29	45/61
Q/total	71/122	0/32	4/29	35/61
DNQ/total	12/122	0/32	2/29	10/61
Median (Log10 copies/ml)	3.01	5.44	1.44	2.67

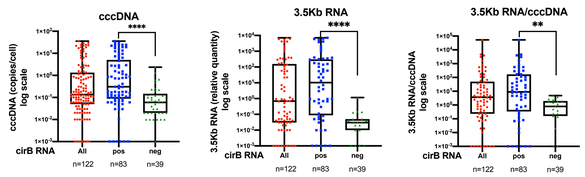
While all HBeAg(+) CH were cirB-RNA(+), 57% of HBeAg(-) CH and only 14% of HBeAg(-) CI patients had quantifiable cirB-RNA. cirB-RNA was higher in HBeAg(+) vs HBeAg(-) patients. Mann-Whitney test, α threshold=0.5. *p<0.05; **p<0.01;***p<0.001; ****p<0.0001. CH=chronic hepatitis; CI=chronic infection; Q=quantifiable; DNQ=detectable not quantifiable.

Figure 1b. …and according to liver disease markers



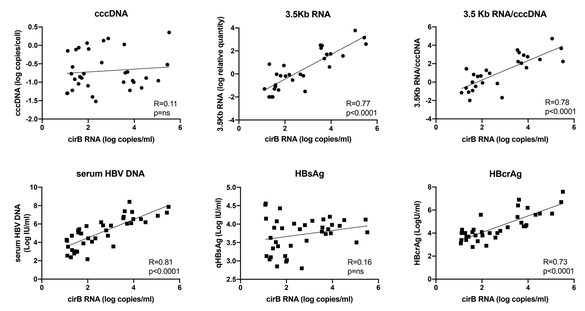
cirB RNA were higher in patients with ALT>2N (5.1 vs 2 log copies/ml) and in those with necroinflammatory activity score (3 vs 1.4 log copies/ml) or fibrosis score ≥1 (3.8 vs 2 Log copies/ml). Mann-Whitney test, α threshold=0.5. *p<0.05; **p<0.01;***p<0.001; ****p<0.0001.

Figure 2. cirB RNA and intrahepatic viral markers



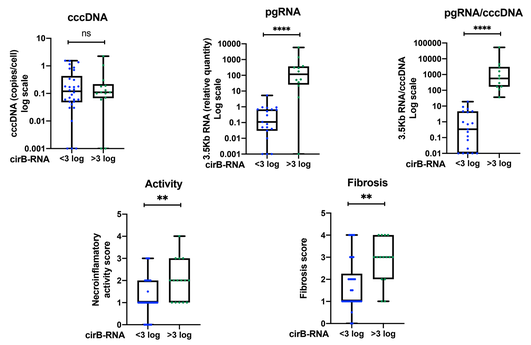
The 39 cirB-RNA(-) patients (23 HBeAg(-) CI and 16 HBeAg(-) CH) had lower cccDNA (median 0.06 vs 0.3 copies/cell), 3.5Kb RNA (median 0.03 vs 10.3) and 3.5Kb RNA/cccDNA (0.79 vs 8.9) as compared to the cirB-RNA(+) ones. Same results were obtained when only HBeAg(-) patients were analyzed. No significant difference was found in qHBsAg levels, while both HBcrAg and serum HBV DNA were significantly higher in cirB-RNA(+) patients (see Table 1). Mann-Whitney test, α threshold=0.5. *p<0.05; **p<0.01;***p<0.001; ****p<0.0001; CH=chronic hepatitis; CI=chronic infection.

Figure 3. Correlations in HBeAg(-) patients



In HBeAg(-) patients, cirB-RNA significantly correlated with serum HBV DNA, HBcrAg, intrahepatic 3.5Kb RNA and cccDNA transcriptional activity, but not with HBsAg and cccDNA levels. Spearman's correlation test, α threshold=0.5.

Figure 4. Two groups of HBeAg(-) CH patients can be discriminated by cirB RNA levels



In HBeAg(-) CH group, a cirB-RNA cut-off >3 log10 copies/ml identified a population of patients with higher intrahepatic 3.5Kb RNA/cccDNA ratio and more advanced liver fibrosis and higher necroinflammatory activity, but without difference in HBsAg levels (3.5 vs 3.9 logIU/ml, p=ns). CH=chronic hepatitis.

CONCLUSION

The Roche IA assay detected HBV RNA in blood circulation (cirB-RNA) in 83/122 patients tested. 74% HBeAg(-) CH and 21% of HBeAg(-) CI scored positive for cirB-RNA

cirB-RNA levels were higher if ALT≥2N and fibrosis or necroinflammatory activity scores were ≥1

Patients with detectable cirB-RNA had higher intrahepatic replicative viral markers, in particular cccDNA and 3.5Kb RNA levels

cirB-RNA positively correlated with intrahepatic cccDNA transcriptional activity, serum HBV DNA and HBcrAg in HBeAg(-) patients

A cirB-RNA threshold of 3 log_{10} copies/ml discriminated HBeAg(-) CH patients harboring higher intrahepatic cccDNA transcriptional activity and fibrosis and necroinflammatory activity scores

Our data strongly support the relevance of cirB-RNA as a non-invasive surrogate marker of intrahepatic cccDNA transcriptional activity and of increased liver damage.

ACKNOWLEDGEMENTS

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For technical details about the Roche IA assay, please refer to Poster 0737

DISCLOSURES

BS, AH, MH are Roche employees. LW was a former Roche employee, now retired.

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