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Authors

King, Wendy
Sterling, Richard
Hinerman, Amanda
et al.

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Hepatitis B RNA and Core-related Antigen Provide Value Beyond DNA in Evaluating e but not Surface Antigen Clearance

Wendy C. King, PhD [Associate Professor of Epidemiology],
Graduate School of Public Health University of Pittsburgh, Pittsburgh, PA, USA

Richard K. Sterling, MD, MSc [Professor and Chief of Hepatology],
Division of Gastroenterology, Hepatology, and Nutrition, Virginia Commonwealth University, Richmond, VA, USA

Amanda S. Hinerman, PhD [Statistician],
Epidemiology department, Graduate School of Public Health University of Pittsburgh, Pittsburgh, PA, USA

Anna S.F. Lok, MD [Professor],
Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, MI, USA

Corresponding Author: Wendy King, PhD, Associate Professor of Epidemiology, Epidemiology Data Coordinating Center, 4420 Bayard Street, Suite 600, Pittsburgh, PA 15260, Phone: 412-901-7728, kingw@edc.pitt.edu.

Contributors: "The HBRN HBV Novel Assays Group"

Abdus S. Wahed, PhD, Professor, Biostatistics department, Graduate School of Public Health University of Pittsburgh, Pittsburgh, PA, USA

Raymond T. Chung, MD, Director of Hepatology and Liver Center, Massachusetts General Hospital, Boston, MA, USA

Harry L.A. Janssen, MD, PhD, Professor, Toronto Centre for Liver Disease, University of Toronto, Toronto, Canada

Mandana Khalili, MD, Professor of Medicine, Department of Medicine, Division of Gastroenterology and Hepatology, University of California San Francisco, San Francisco, CA, USA

Daryl T.Y. Lau, MD, Associate Professor, Division of Gastroenterology and Hepatology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

William M Lee, MD, Meredith Mosle Chair in Liver Disease, UT Southwestern Medical Center, Dallas, TX, USA

Mauricio Lisker-Melman, MD, Professor, Washington University School of Medicine and John Cochran VA Medical Center, St. Louis, MO. USA

Norah Terrault, MD, MPH, Professor, Division of Gastrointestinal and Liver Diseases, Keck Medicine of University of Southern California, Los Angeles, CA, USA

Author contributions

Wendy C King performed the data analysis, contributed to analysis and interpretation of results, drafted the manuscript, and revised the manuscript critically for content. Amanda S Hinerman performed the data analysis, contributed to analysis and interpretation of results, and revised the manuscript critically for content. Anna SF Lok and Richard K Sterling contributed to data collection, analysis and interpretation of results, and reviewed and revised the manuscript critically for content. Gavin A Cloherty contributed to creation of exploratory HBV RNA assay, generation of data, analysis and interpretation of results and reviewed and revised the manuscript critically for content. Marc Ghany contributed to data collection, analysis and interpretation of results, drafted the manuscript and revised critically for content. All authors approved the final manuscript for submission.

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Gavin A Cloherty, PhD [Head of Infectious Disease Research],
Abbott Diagnostics, Abbott Park, USA

Marc G. Ghany, MD, MS [Investigator],
Liver Diseases Branch, NIDDK, NIH, Bethesda, MD, USA.

The HBRN HBV Novel Assays Group (Corporate authorship)

In chronic hepatitis B virus (HBV) infection, hepatitis B e antigen (HBeAg) and hepatitis B surface antigen (HBsAg) clearance are important milestones towards immune control[1]. A drop in HBV deoxyribonucleic acid (DNA) is an established correlate of both HBeAg and HBsAg clearance[2]. We evaluated changes in HBV ribonucleic acid (RNA) and hepatitis B core-related antigen (HBcrAg) levels, markers of transcriptional activity of covalently closed circular DNA[3,4] (cccDNA), with HBeAg and HBsAg clearance, and compared them to changes in HBV DNA level among adult participants in the Hepatitis B Research Network (HBRN).

The HBRN Adult Cohort study (NCT01263587) enrolled untreated HBsAg-positive adults from 28 sites across North America[5]. Following study entry, HBV treatment could be initiated/stopped by their physician. Study assessments, conducted every 24 weeks, included medical history, physical examination, health surveys, routine blood tests and HBV serologies[6]. The study protocol was approved by sites' institutional review boards. Participants provided written informed consent.

Two nested case-control samples were identified for this report. Cases were HBeAg-positive or HBsAg-positive participants with confirmed HBeAg or HBsAg clearance (≥2 positive results followed by ≥2 negative results) who had stored serum samples 48±12 weeks before and 24 ± 12 weeks after their first negative HBeAg or HBsAg result. Controls were sex and age (±5 years) matched HBeAg-positive or HBsAg-positive participants, respectively, with serum samples available 72 (±24) weeks apart with consecutive positive results and no negative results during this timeframe. This resulted in 50 participants (25 pairs) in the HBeAg sample and 40 participants (20 pairs) in the HBsAg sample.

Qualitative and quantitative HBeAg and HBsAg were determined using the Roche Diagnostics Elecsys platform with lower limits of detection (LLOD) of 0.3 IU/mL for HBeAg and 0.05 IU/mL for HBsAg[7,8]. HBV DNA levels were determined using a real-time PCR assay with LLOD of 10 IU/mL and lower limit of quantification (LLOQ) 20 IU/mL (COBAS Ampliprep/COBAS TaqMan HBV Test, v2.0; Roche Molecular Diagnostics, Branchburg, NJ). HBV RNA was quantified using the RealTime HBV RNA Research Use Only (RUO) assay on the Abbott m2000 system (Department of Infectious Diseases, Abbott Diagnostics, Abbott Park, USA), with a LLOQ of 1.65 $\log_{10}$ U/ml. HBcrAg concentrations were measured using a RUO chemiluminescence enzyme immunoassay (Lumipulse G®, Fujirebio, Gent, Belgium) with a LLOD of 3.0 $\log_{10}$ U/ml and linear range of 3.0-<6.8 $\log_{10}$ U/ml[9]. Additional information on HBV biomarkers is provided in the Appendix.

A series of mixed-effects binomial regression models fit via maximum likelihood were used to evaluate associations between each HBV biomarker (continuous, $\log_{10}$) at baseline

and the change from baseline, respectively, with HBeAg and HBsAg clearance status, respectively, with time as a continuous fixed effect and matching ID as a random effect. Modeling was repeated with adjustment for baseline alanine aminotransferase (ALT) x upper limit of normal (ULN; males 35 U/L, females 20 U/L) and HBV treatment (≥24 weeks in the past 36 weeks[2]) at time of outcome assessment.

Baseline characteristics overall and by clearance status of both samples are provided in sTable 1. Among the HBeAg sample, 28% were treated,10% had ALT >5xULN and none had HBV RNA and HBcrAg values below quantification, whereas 22% had HBV DNA values below quantification, most of whom were treated. Lower baseline values of all 3 HBV markers were associated with higher odds of HBeAg clearance; HBcrAg had the best model fit, followed by HBV RNA (Table 1).

Among HBeAg controls, HBV DNA, HBV RNA and HBcrAg were relatively stable across follow-up (median decreases were 0.2, 0.1 and 0.3 $\log_{10}$ U/ml, respectively), whereas cases had median decreases of 0.4 $\log_{10}$ IU/ml HBV DNA, 1.6 $\log_{10}$ U/ml HBV RNA and 0.9 $\log_{10}$ U/ml HBcrAg (sFigure 1, panel A-C). Decreases in HBV RNA and HBcrAg, but not change in HBV DNA, were associated with higher odds of HBeAg clearance; change in HBV RNA had the best model fit (Table 1).

Among the HBsAg sample, at baseline, none were treated, 5% were HBeAg positive and 3% had ALT >5xULN (sTable 1). Forty-eight percent, 20% and 15% had quantifiable baseline values of HBV DNA, HBcrAg, and HBV RNA, respectively. Lower baseline values of HBV DNA and HBV RNA, but not HBcrAg, were associated with higher odds of HBsAg clearance; HBV RNA had the best model fit (Table 1).

Among HBsAg controls, HBV DNA, HBV RNA and HBcrAg were relatively stable across follow-up (median decreases of 0.1, 0.3 and 0.1 $\log_{10}$ U/ml, respectively). HBsAg cases had a median decrease of 0.5 IU/ml HBV DNA, whereas HBV RNA and HBcrAg values were stable (median change of 0.0 $\log_{10}$ U/ml for both) (sFigure 1, panel D-F). Decreases in HBV DNA, but not change in HBV RNA or HBcrAg, was associated with higher odds of HBsAg clearance (Table 1). Adjustment for ALT or HBV treatment did not change the relative performance of HBV biomarkers with regard to HBeAg or HBsAg clearance (Table 1).

Clinical and traditional virologic markers and RUO quantitative HBsAg accurately predict HBeAg and HBsAg clearance in HBV[2,10]. However, we showed that compared to HBV DNA, baseline HBcrAg better predicted HBeAg clearance, likely because HBeAg is the predominant protein measured in the HBcrAg assay among HBeAg positive persons. In contrast, HBcrAg did not predict HBsAg clearance, while baseline HBV RNA and HBV DNA had similar predictive ability.

Evaluation of changes in HBV biomarkers during HBeAg and HBsAg clearance revealed: 1) HBeAg clearance resulted in greater relative declines in HBV RNA and HBcrAg compared to HBV DNA, suggesting that HBeAg clearance is associated with a greater inhibition of viral transcription than replication; 2) HBsAg clearance resulted in further decline in HBV DNA but no observable change in HBV RNA or HBcrAg, likely because cccDNA was

transcriptionally silent at baseline (indicated by unquantifiable HBV RNA and HBcrAg values) in most who cleared HBsAg approximately 48 weeks later.

In conclusion, our study highlights the value of monitoring cccDNA transcriptional activity via HBV RNA and HBcrAg as a predictor of HBeAg clearance. Additionally, it demonstrates that cccDNA transcriptional activity markedly declines after HBeAg clearance, and cccDNA transcriptional inactivity is a pre-requisite for HBsAg clearance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Odds of HBeAg or HBsAg clearance by HBV serum marker (at baseline or change between time points[a])

	Odds ratio of clearance per 1 unit (log_{10}) lower					
	Unadjusted OR (95% CI)	P	BIC[b]	Adjusted[c] OR (95% CI)	P	BIC[b]
Outcome: HBeAg Clearance	**N=50**			**N=48**		
Baseline						
HBV DNA (log_{10} U/mL)	1.59 (1.22–2.09)	0.002	60.7	1.58 (1.03–2.41)	0.04	63.9
HBV RNA (log_{10} U/mL)	2.37 (1.44–3.92)	0.002	57.1	2.71 (1.33–5.52)	0.009	57.0
HBcrAg (log_{10} U/mL)	3.45 (1.71–6.96)	0.001	52.9	6.22 (1.61–24.04)	0.01	49.6
Change (follow-up-baseline)						
HBV DNA (log_{10} U/mL)	1.06 (0.83–1.36)	0.60	77.7	1.05 (0.75–1.48)	0.76	68.9
HBV RNA (log_{10} U/mL)	2.99 (1.35–6.61)	0.009	62.4	4.85 (1.61–14.55)	0.007	54.0
HBcrAg (log_{10} U/mL)	2.08 (1.16–3.73)	0.02	68.8	2.75 (1.01–7.47)	0.048	62.8
Outcome: HBsAg Clearance	**N=40**			**N=38**		
Baseline						
HBV DNA (log_{10} IU/mL)	10.43 (2.17–50.06)	0.006	45.2	11.42 (1.92–68.00)	0.01	50.8
HBV RNA (log_{10} U/mL)	18.84 (2.12–167.66)	0.01	43.9	21.42 (1.97–232.93)	0.02	50.0
HBcrAg (log_{10} U/mL)	1.29 (0.80–2.08)	0.27	62.7	1.36 (0.71–2.60)	0.33	66.3
Change (follow-up-baseline)						
HBV DNA (log_{10} IU/mL)	4.88 (1.37–17.35)	0.02	54.1	8.93 (1.60–49.81)	0.02	55.0
HBV RNA (log_{10} U/mL)	0.90 (0.37–2.20)	0.81	64.0	0.86 (0.32–2.30)	0.75	67.2
HBcrAg (log_{10} U/mL)	0.96 (0.61–1.49)	0.84	64.0	0.95 (0.58–1.57)	0.84	67.2

Acronyms: BIC, Bayesian information criterion ; DNA, deoxyribonucleic acid; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; OR, odds ratio; RNA, ribonucleic acid

[a]Baseline was approximately 48 weeks prior to outcome assessment. Follow-up was approximately 72 weeks later.

[b]A lower BIC is good evidence of a better model fit when comparing models for the same outcome with the same number of independent variables.

[c]Adjusted for baseline ALT x ULN and HBV treatment ≥24 weeks in past 36 weeks prior to outcome assessment (HBV treatment use was 68% among cases and 20% among controls in the HBeAg sample; 0% among cases and 5% among controls in the HBsAg sample). One pair (n=2 participants) for each outcome was excluded from the model due to missing baseline ALT.