nature portfolio

https://doi.org/10.1038/s41575-022-00649-z

Supplementary information

A roadmap for serum biomarkers for hepatitis B virus: current status and future outlook

In the format provided by the authors and unedited

Supplementary Table 1. Key features of classic serum biomarkers: HBV DNA, HBeAg and/ or anti-HBe, HBsAg, anti-HBs and anti-HBc

[a]Source[1]	Clinical Relevance During Natural Infection	Clinical Relevance Following Treatment	[b]Detection
HBV DNA			
Derived following reverse transcription of HBV pgRNA, exclusively transcribed from cccDNA; found intracellularly in capsids and extracellularly in infectious virions	Hallmark of infection; Highest HBV DNA levels in high replicative and low inflammatory phase, decreasing during immune clearance[1]	Treatment-induced suppression correlates with lower risk of liver disease progression and HCC[2]; during NUC treatment, serum HBV DNA suppression is incomplete, cccDNA persistence can lead to relapse following NUC cessation[3]; resistance to NUCs can lead to increased HBV DNA in serum[4]	Commercially available assays: Abbott realtime HBV LLOD 10 IU/mL (0.5 mL serum); 15 IU/mL (0.2 mL serum) (quantitative, platform); Abbott Alinity m LLOQ 10 IU/ml, LLOD 9.6 IU/mL (serum); 6.7 IU/mL (plasma) (quantitative, platform); Roche Cobas LLOD 2.4 IU/mL (serum); 2.7 IU/mL (plasma) (quantitative, platform); Roche 6800 LLOQ 10 IU/ml (quantitative, platform); Cepheid Xpert LLOQ 10 IU/ml; LLOD 3.2 IU/mL (plasma), 6.0 IU/mL (serum) (quantitative, platform)
HBeAg and/or anti-HBe			
Immunomodulatory protein[5,6] translated from preC mRNA[7,8]; HBeAg appears early during infection, with anti-HBe antibodies appearing later	HBeAg status defines CHB phases (HBeAg-positive or negative infection)[9]; positivity correlates with high viremia, but negativity does not exclude liver disease[10]; transition to HBeAg-negative CHB is associated with a reduction in cccDNA levels, transcriptional activity, and HBV DNA levels[11]; HBeAg loss is associated with reduced disease activity and favourable long-term outcome[12]; higher titres of anti-HBe, with anti-HBc IgG, might reflect cccDNA activity in HBsAg-negative CHB[13]	HBeAg loss and anti-HBe seroconversion can be an indication of virological remission, but the development of basal core promoter and/or precore mutations can lead to HBeAg-negativity with high viral loads[14]; HBeAg to anti-HBe seroconversion identifies a primary endpoint in peg-IFNα and NUC treatment, with discontinuation in patients who are HBeAg positive[9]; anti-HBe antibodies might influence the risk of viral reactivation after treatment discontinuation in patients who are HBeAg negative[15]	Commercially available assays: Roche Elecsys HBeAg assay (qualitative, ECLIA); Diasource LLOD 0.59 PE IU/mL; (qualitative, ELISA) DiaSorin LLOD 0.30 PE IU/mL (qualitative); Biorad Monolisa LLOD 0.64 PE IU/mL (qualitative); Abbott Alinity i LLOD 0.144 – 0.157 PE IU/mL (qualitative, platform); Abbott Architect LLOD ≤0.5 PE IU/mL (qualitative, CLIA); DiaSorin LIAISON® XL MUREX, HBeAg, LLOQ 0.10 PE IU/mL (quantitative, CLIA) anti-HBe assays: Diasource LLOD 0.30 PE IU/mL (qualitative, ELISA); Biorad Monolisa LLOD 2.50 PE IU/mL (qualitative, ELISA); Roche Elecsys LLOD <0.2 WHO IU/mL (qualitative,

			ECLIA); Abbott Alinity i LLOD 0.14 – 0.15 IU/mL (qualitative, CMIA); Abbott Architect LLOQ ≤0.45 PE IU/mL (quantitative, CMIA); DiaSorin LIAISON® XL MUREX, , LLOQ ≤0.076 PE IU/mL (quantitative, CLIA); Lumipulse®G600II LLOD 0.31 IU/mL and LLOQ 0.35 IU/mL (qualitative and quantitative, CLEIA, CLIA)[13]
HBsAg			
A group of three proteins expressed from both cccDNA and integrated HBV DNA and embedded into the envelope of the infectious virions or forming spherical and filamentous subviral particles, which are non-infectious [16,17]	Used to establish prevalence of HBV infection; CHB is diagnosed by a positive repeat test for HBsAg; highest HBsAg levels in HBeAg-positive, high replicative CHB phase, reducing in HBeAg-negative CHB and low replicative phases[1]; HBsAg titres modestly correlate with cccDNA levels and only in HBeAg-positive CHB[18], which might direct most of the HBsAg production during HBeAg-negative CHB[19]; HBsAg seroclearance occurs annually in 0.1–2% of patients with CHB[9,15]; HBsAg level is a better predictor for HBsAg clearance than HBV DNA levels[18,20,21]; HBsAg level can predict HBsAg seroclearance during natural disease progression or with current therapies[22,23]; multiple virus and host factors can affect HBsAg production and secretion[24]; the relationship between HBsAg and cccDNA levels differs between studies, particularly in	HBsAg is a stable endpoint for off-treatment sustained response for NUCs and peg-IFNα[25,33]; HBsAg loss is rare but indicates immune control of viral replication associated with histological improvement[34]; NUCs have limited effect on HBsAg levels in patients who are treated[35,36]; low serum HBsAg levels might indicate low cccDNA levels and/or integrated HBV DNA and are the best predictor to date of the likelihood of relapse following treatment cessation[37-39]; ≥1 $\log_{10}$ IU/ml decline in HBsAg levels by week 12 of peg-IFNα treatment is a strong predictor of sustained response[18,40,41]; peg-IFNα treatment can induce substantial epigenetic suppression of cccDNA transcription even in the absence of immune responses[42-44]; reduction of HBV transcripts including HBx mRNA and reactivation of the host SMC5/6 complex[45,46] might have a key role in cccDNA silencing[44,47]; newer treatments using small interfering RNA, can reduce HBsAg levels[48], sometimes accompanied by transient ALT flares[49]	Commercial assays: Diasource LLOD 0.20 ng/mL (qualitative, ELISA); Biorad Monolisa LLOD 0.06 ng/mL (qualitative, ELISA); Abbott Alinity i NEXT LLOD 4.50 – 5.97 nIU/mL (NIBSC 00/588, 2003) (qualitative, CLIA); Abbott Alinity i v2.0 LLOD 0.02 – 0.021 IU/mL (NIBSC 00/588, 2003) (qualitative, CLIA); Abbott Alinity i QT LLOQ 0.05 IU/mL (quantitative, CMIA); Abbott Architect v2 LLOD 0.014 – 0.049 IU/mL; 0.05 IU/mL (NIBSC 00/588, 2003) (qualitative, CLIA); Abbott Architect QT LLOQ 0.05 IU/mL; (quantitative, CMIA); Roche Elecsys LLOQ 0.04 PE IU/mL (quantitative, CLIA); DiaSorin LIAISON® XL MUREX, HBsAg, LLOQ 0.05 IU/mL**;** 0.05 IU/mL (NIBSC 00/588, 2003) (quantitative, CLIA); DiaSorin Murex v3 (qualitative, ELISA)

	patients who are HBeAg-negative [18,19,25]; HBsAg loss is associated with better clinical outcome: less cirrhosis, HCC and mortality[26]; the predictive potential of HBsAg levels for the development of HCC is enhanced when combined with HBV DNA levels[27-29]; in patients who are HBeAg−negative with low viral loads, higher HBsAg levels independently predict HCC development[30]; minimal risk individuals can be predicted using: qHBsAg < 1000 IU/mL + low HBV DNA (<2000 IU/mL) plus normal ALT[23,30,31]; qHBsAg levels are excluded from most HCC prediction models except for the “REACH-B” model[32];		
Anti-HBs			
Neutralizing antibodies directed against HBsAg; titres > 10 mIU/ml correlate with protection following prophylactic vaccination; levels can be underestimated in CHB because of antigen-antibody complexes with high circulating HBsAg[50]	Indicative of immunity to HBV following natural infection or vaccination and recovery of acute HBV infection; in occult HBV infection, anti-HBs seems to influence the risk of reactivation in case of immunosuppression[51]; immune complexes at baseline associated with ALT flare and functional cure on therapy, to date, findings are limited to HBV genotype A[52]	Might or might not be present following HBV DNA and/or HBsAg loss following treatment.	Commercially available assays: Diasource LLOD 3.6 mIU/mL (qualitative, ELISA); Biorad Monolisa LLOD 2 mIU/mL (qualitative,ELISA); Roche Elecsys-anti-HBsII LLOD 2 IU/L; range 2 – 1000 IU/L; < 10 IU/L non-reactive; ≥ 10 IU/L reactive (quantitative, ECLIA); Abbott Alinity i LLOD not specified ≥ 12.00 mIU/mL, grayzone ≥ 8.00 - < 12.00 mIU/mL (qualitative, CMIA); Abbott Architect LLOQ not specified, reactive 10 mIU/mL (quantitative, CLIA); DiaSorin LIAISON® LLOD 3 mIU/mL (quantitative, CLIA)

Anti-HBc			
Non-neutralizing antibodies are directed against the viral capsid.	Biomarker of natural exposure to the virus; total anti-HBc is a marker of acute, chronic, and resolved HBV infection, or occult hepatitis B; anti-HBc IgM is detected during acute HBV infection and exacerbations of CHB; anti-HBc can be a predictor of liver inflammation, particularly when used together with serum HBsAg in patients who are HBeAg-positive or HBeAg-negative[53]	Anti-HBc can predict HBV reactivation following immunosuppression [51]; anti-HBc measurement is recommended for diagnosis in patients suspected to have an acute exacerbation of CHB or to decide if patients who are planning to undergo immunosuppressive therapy will require prophylactic antiviral therapy[1]; quantitative anti-HBc (IgG or total) might be useful to better characterize the risk of reactivation after NUC discontinuation, the risk of HCC occurrence and that of HBV reactivation in HBsAg negative immunosuppressed individual[54,55]	Anti-HBc total assays: Diasource LLOD 1.87 PE IU/mL (qualitative, ELISA); Biorad Monolisa LLOD 0.5 PE IU/mL for IgG and 8 PE IU/mL for IgM (qualitative, ELISA); Roche Elecsys LLOD 0.8 WHO IU/mL (qualitative, ECLIA); Abbott Alinity i LLOD 0.61 IU/mL (qualitative, CMIA); Abbott Alinity i v2 LLOD 0.54 – 0.56 IU/mL (qualitative, CMIA); Abbott Architect V2 LLOQ $\leq$1 PE IU/mL (quantitative, CMIA); DiaSorin LIAISON® LLOD 0.6 PE IU/mL (qualitative, CLIA); DiaSorin Murex (total) (qualitative, ELISA) Anti-HBc IgM assays: Diasource LLOD 2.5 PE IU/mL (qualitative, ELISA); Biorad Monolisa LLOD 50 PE IU/mL (qualitative, ELISA); Roche Elecsys LLOD $\leq$3 PE IU/mL (qualitative, ECLIA); Abbott Architect LLOQ 0.4 – 0.5 PE IU/mL (quantitative, CMIA);

			DiaSorin LIAISON® LLOD 20 PE IU/mL (qualitative, CLIA);

[a]For details on the role of the markers in the viral life cycle and their expression during natural infection please refer to Yuen et al., 2018[1]

[b]The list of commercially available kits is far from exhaustive but represents the commonly used platforms or assays.

ALT: alanine aminotransferase; anti-HBc: antibody against HBcAg; anti-HBe: antibody against HBeAg; cccDNA: covalently closed circular DNA; CHB: chronic hepatitis B; CLEIA: chemiluminescent enzyme immunoassay; CLIA: chemiluminescent immunoassay; CMIA: chemiluminescent microparticle immunoassay; DNA: deoxyribonucleic acid; ECLIA: Electrochemiluminescence immunoassay; ELISA: enzyme-linked immunosorbent assay; HBcAg: hepatitis B core antigen (capsid protein); HBeAg: hepatitis B virus e antigen; HBsAg: hepatitis B s antigen (envelope protein); HBV: hepatitis B virus; HBx: hepatitis B x protein; HCC: hepatocellular carcinoma; IgG: immunoglobulin G; IgM: immunoglobulin M; IU: international units; LLOD: lower limit of detection; LLOQ: lower limit of quantitation; mRNA: messenger ribonucleic acid; NIBSC: National Institute for Biological Standards and Control; NUC/s: nucleos(t)ide analogue/s; PE: Paul Ehrlich; peg-IFNα: pegylated interferon-α; pgRNA: pregenomic RNA; preC: precore; qHBsAg: quantitative hepatitis B s antigen; SMC5/6: structural maintenance of chromosomes; WHO: World Health Organization

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