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A comparison of two assays for quantification of Hepatitis B surface Antigen in patients with chronic hepatitis B

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ABSTRACT

Background and objectives: Serum Hepatitis B surface Antigen (HBsAg) levels correlate with hepatitis B virus intrahepatic covalently closed circular DNA and may predict response to treatment. Currently, 2 commercial platforms are available for HBsAg quantification in clinical practice, the Architect HBsAg QT and the Elecsys HBsAg. We aimed to directly compare the results of these assays.
Study design: HBsAg levels were measured in 1427 serum samples from HBeAg-positive chronic hepatitis B patients who participated in a randomized trial of peginterferon alfa-2b ± lamivudine. Samples were extracted from our serum bank, thawed, and subsequently analysed for HBsAg levels using both assays.
Results: Of 1427 samples, 242 (17%) were taken before and 1185 during the treatment phase of the study. Distribution of HBV genotypes was 447 (31%) genotype A, 125 (9%) B, 210 (15%) C and 534 (37%) D. Correlation between Architect and Elecsys results was high ($r = 0.96$, $p < 0.001$). By Bland–Altman analysis, agreement between the two assays was close (mean difference between Architect and Elecsys: −0.01 log IU/mL, 95% CI: −0.55–0.52 log IU/mL), also when analysed separately for HBV genotypes A–D. Additionally, the performance of our recently published stopping rule for HBeAg-positive patients treated with peginterferon was comparable: the negative predictive values were 96% and 98% for Elecsys and Architect, respectively.
Conclusions: There is a high correlation and close agreement between quantitative HBsAg measurements conducted with the Architect and the Elecsys. Clinical prediction rules derived from data from one platform can be applied on the other; both can therefore be used in clinical practice.

1. Background and objectives

Chronic hepatitis B (CHB) is an important global health problem, with over 350 million people being chronically infected.[1] Hepatitis B surface Antigen (HBsAg) is an established marker of infection with the hepatitis B virus (HBV), and is therefore often used as a screening tool.[1] In addition to its use as a qualitative marker, recent innovations have allowed for the quantitative assessment of HBsAg in serum. The clinical relevance of HBsAg levels is derived from its correlation with intrahepatic HBV covalently closed circular (ccc) DNA, the main replicative template of HBV.[2,3] Through this association, serum HBsAg is hypothesized to be a marker for immunological response to therapy, independent of virological response as measured using HBV DNA levels.

Several clinical applications of HBsAg levels have been described. For example, HBsAg levels appear to differentiate patients in the inactive carrier state (persistent HBeAg negativity, with HBV DNA <2000 IU/mL, normal ALT[4]) from those with active disease or from inactive carriers with a high probability of subsequent relapse.[5–7] Furthermore, recent studies have shown that on-treatment HBsAg levels are predictive of a durable off-treatment response to peginterferon (PEG-IFN), and can accurately identify non-responders early during therapy, both in HBeAg-positive,[8–10] and HBeAg-negative[11,12] patients.

Currently, multiple diagnostic assays are available for quantification of HBsAg. The most widely used is the Architect assay,[13] but HBsAg quantification may also be performed using the Elecsys

Abbreviations: CHB, chronic hepatitis B; HBsAg, Hepatitis B surface Antigen; HBV, hepatitis B virus; cccDNA, covalently closed circular DNA; PEG-IFN, peginterferon; HBeAg, hepatitis B e antigen.

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platform (HBsAg Quant Package Insert, Roche Diagnostics 2011). The comparability of the two assays in measuring HBsAg levels in patient sera is however unknown. The aim of our study was therefore to compare quantitative HBsAg measurements conducted with the Architect HBsAg QT assay with measurements conducted with the Elecsys HBsAg in a large number of patient samples.

2. Study design

2.1. Assays

HBsAg was quantified using the Architect platform (Abbott Laboratories, Abbott Park, IL, USA) and Elecsys HBsAg (Roche Diagnostics, Indianapolis, IN, USA). HBsAg testing was performed according to the manufacturer's package insert and with test kits from a single lot. For the Architect, sample material and anti-HBs-coated paramagnetic microparticles are combined. After a washing step, acridinium labelled anti-HBs conjugate is added, and after another washing cycle, pre-trigger and trigger solutions are added. The subsequent chemiluminescent reaction is measured in relative light units, which can be converted directly to HBsAg units. The range of the assay is 0.05–250 IU/mL, and if results were outside of this range, material was diluted according to the manufacturer's recommendations (package insert HBsAg, Abbott Laboratories 2008). The Elecsys HBsAg assay is an immunoassay that measures HBsAg using a sandwich principle: first a complex is formed with two monoclonal HBsAg-specific antibodies, one of which is biotinylated, the other labelled with a ruthenium complex. After addition of streptavidin-coated microparticles, the complexes bind to the solid phase through interaction of biotyn and streptavidin. The mixture is subsequently aspirated into a measuring cell, where application of a voltage then induces chemiluminescent emission, which is measured by a photomultiplier. The measured results are compared to a cut-off value obtained through HBsAg calibration. The obtained signal to cut-off index may then be converted to IU/mL using a WHO standard conversion factor of 0.055 IU/mL (all methodology as per Elecsys package inserts for quantitative HBsAg measurement, Roche Diagnostics 2011). All assays were performed at the Erasmus MC University Medical Center in Rotterdam, the Netherlands.

2.2. Samples

Samples for this study were derived form a multicenter randomized trial investigating the efficacy of 52 weeks of PEG-IFN alfa-2b ± lamivudine for the treatment of HBeAg-positive CHB.[14] In- and exclusion criteria for this study have been previously published.[14] Samples were stored after the study in a large serum bank. For this study, samples were thawed and serum was extracted for quantitative HBsAg measurements using the two assays. HBV genotype was assessed using the INNO-LiPA line-probe assay.[15]

2.3. Statistical analysis

After transformation to log(10) IU/mL, the results of the two assays were compared using correlation (Pearson) and Bland–Altman analyses. A p value of <0.05 was considered statistically significant. Additionally, the results of a previously reported stopping rule for PEG-IFN therapy, established using the Architect assay,[10] were applied on the Elecsys data for comparison.

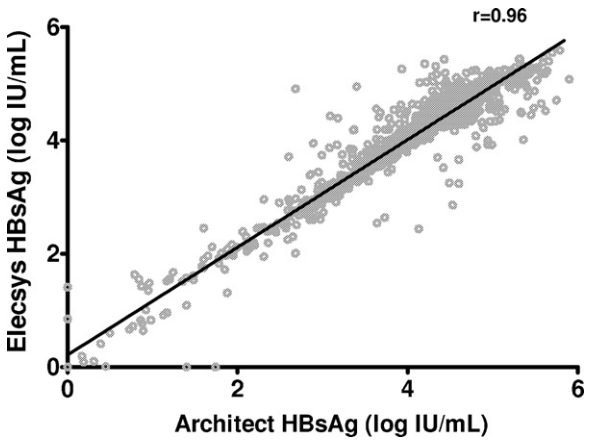

Fig. 1. Correlation between HBsAg measurements using the Architect HBsAg QT assay and the Elecsys HBsAg.

3. Results

3.1. Samples

A total of 1427 samples were measured using both assays, of which 242 (17%) were samples taken before treatment and 1185 (83%) were taken during the treatment phase. The samples retested for this study represent a random subset of the original study population.[10]

3.2. Comparison between Architect and Elecsys measurements

A correlation of measurements conducted with the two assays is shown in Fig. 1; correlation between the assays was excellent ($r = 0.96$, $p < 0.001$). Using regression analysis, it was determined that $HBsAg_{Architect} = 0.979 * (HBsAg_{Elecsys}) + 0.074$. Bland–Altman analysis (depicted in Fig. 2) revealed a close agreement between the two tests: the results of the Elecsys were on average only 0.01 log IU/mL higher than the Architect assay. Overall, the range for the mean difference between the two test results (±2 standard deviations) was −0.55 through 0.52 log IU/mL. Additionally, in 83.4% of paired samples the difference between the two assays was <0.25 log IU/mL, in 93.6% <0.5 log IU/mL and in 98.2% the difference between the two test results was <1 log IU/mL.

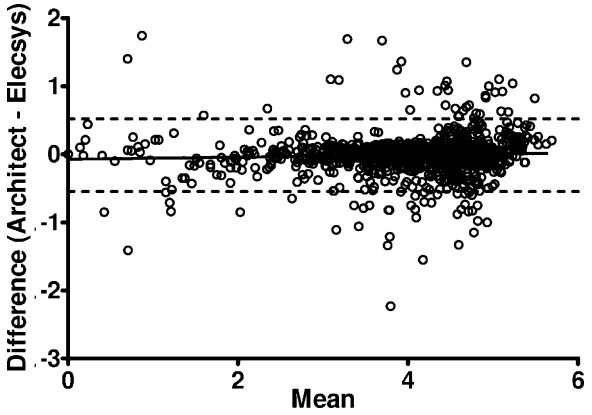

Fig. 2. Bland–Altman plot of HBsAg measurements using the Architect HBsAg QT assay and the Elecsys HBsAg. Dashed lines represent 95% confidence limits.

Table 1
Bland–Altman analysis per genotype.

Genotype	Bias	95% limits of agreement	
		−2SD	+2SD
A (n = 447)	−0.051	−0.555	0.454
B (n = 125)	0.063	−0.414	0.540
C (n = 210)	−0.053	−0.387	0.281
D (n = 534)	0.011	−0.610	0.631

Overview of the results of the Bland–Altman comparison of the Architect versus the Elecsys HBsAg data in samples from patients with HBV genotypes A, B, C and D. SD, Standard deviation.

3.3. Comparison according to HBV genotype

Of the 1427 samples, 447 (31%) were taken from patients with HBV genotype A, 125 (9%) from patients with genotype B, and 210 (15%) and 534 (37%) from patients with genotypes C and D, respectively. The remaining samples (n = 111, 8%) were from patients who harboured different or mixed HBV genotypes, and were not considered for this part of the analysis. The results of the two assays were comparable, within narrow confidence limits, irrespective of HBV genotype (Table 1).

3.4. Retesting of samples with high divergence

A divergence of ≥1 log IU/mL was found in 25 samples (1.8%). High divergence was not related to HBV genotype or treatment week. Sufficient serum for retesting on both platforms was available in 20 (80%). The test results observed in the retested samples were highly correlated (0.99, $p \leq 0.001$), and the mean difference between the results for the Architect when compared to the Elecsys was −0.10 log IU/mL, with none of the samples with detected HBsAg diverting ≥0.5 log IU/mL. The mean differences between first and second measurement were −0.02 log IU/mL for the Architect and 0.02 log IU/mL for the Elecsys.

3.5. Comparison of the performance of a threshold based stopping-rule

Recently, we have reported a stopping-rule for HBeAg-positive patients treated with PEG-IFN, based on the presence or absence of a decline in serum HBsAg levels from baseline. Patients without a decline from baseline to week 12 of treatment ($\log(\text{HBsAg}_{\text{week12}}) - \log(\text{HBsAg}_{\text{baseline}}) \geq 0$) had a very low probability of response (<5%), and we advised to discontinue therapy in these patients.[10] For the current study, we applied this threshold based rule on the subset of patients that had both Architect and Elecsys data available. For this analysis, a representative subset of 181 patients of the original study (out of 221, 82%) were included. As shown in Table 2, the stopping rule performed very well within the subset of patients included in this analysis, and the positive predictive values (PPVs) and negative predictive values (NPVs) obtained with the two assays were very similar (NPVs: 96% for the Elecsys, compared to 98% for the Architect).

Table 2
Positive and negative predictive values of the stopping-rule.

Response	Architect				Elecsys			
	No	Yes	PPV	NPV	No	Yes	PPV	NPV
Any decline week 12								
Yes	97	30	24%	–	101	29	22%	–
No	53	1	–	98%	49	2	–	96%

Response was defined as HBeAg loss and HBV DNA < 10,000 copies/mL 6 months after discontinuation of treatment. NPV, negative predictive value; PPV, positive predictive value.

4. Discussion

This is the first large study comparing the two major commercial platforms, Architect and Elecsys, for HBsAg quantification in patient sera. We found a very close agreement between the assays, irrespective of HBV genotype. Both assays can therefore be used for HBsAg quantification in clinical practice in HBeAg-positive patients.

Presence of HBsAg is commonly used as a screening tool for infection with HBV, and is often detectable in serum before evidence of liver inflammation is present.[16] However, recent technological advancements have allowed for the quantification of HBsAg in serum. The clinical relevance of these HBsAg levels is derived from the apparent correlation with intrahepatic cccDNA levels, which are strong predictors of sustained response to treatment, but can only be assessed invasively through a liver biopsy.[3,17]

Recent studies have shown that HBsAg levels may be used in the monitoring of HBV therapy. During therapy with nucleos(t)ide analogues (NA), HBV DNA is potently suppressed to undetectable levels in a majority of patients after 48 weeks of treatment, but only moderate declines in HBsAg levels are achieved.[8,11,18] HBsAg and HBV DNA therefore seem largely uncorrelated during antiviral therapy, and HBsAg decline may signify an immunological response that is independent of HBV DNA suppression as achieved with NA based treatment. Monitoring of HBsAg levels during antiviral therapy for CHB may therefore provide additional information when compared to HBV DNA levels alone. One year of PEG-IFN therapy induces a pronounced decline in HBsAg levels, especially in patients who achieve an off-treatment sustained response.[8,11,12,19,20] The differences in HBsAg decline between responders and non-responders can be used to predict success of therapy.[10,12,20] Additionally, HBsAg decline during therapy with NA may be used to predict subsequent loss of HBsAg through prolonged follow-up.[21,22]

Most of the recent studies on HBsAg quantification were conducted using the Architect platform, but the manual for HBsAg quantification on the Elecsys was recently released (HBsAg Quant, Roche Diagnostics 2011). The comparability of the results of the two assays was however still unknown, and the limited insight into the agreement between the two assays prohibited extrapolation of data and findings from studies using either platform to studies or clinics that use the other. The current study was designed to address these issues, and we have now shown that the two assays provide comparable results. Importantly, there is a very close agreement between the two platforms, within ±0.5 log IU/mL in 94% of the samples that were tested, and this close agreement is independent of HBV genotype.

In addition to the use of HBsAg in monitoring therapy efficacy, several reports have now provided stopping-rules for PEG-IFN therapy for CHB. A post hoc analysis of the phase 3 study of PEG-IFN alfa-2a showed that lower HBsAg levels during treatment were associated with a sustained off-treatment response.[8] Similarly, we showed that HBeAg-positive patients without a decline at week 12 of therapy had a low probability of sustained response, and no chance of HBsAg loss, even through long term follow-up.[10] The current study shows that our findings reported from data gathered using the Architect can be extrapolated to measurements conducted with the Elecsys. This implies that published threshold or decline based prediction rules established using either platform may be used on the other as well, without losing predictive accuracy. Furthermore, our findings can also be extended to measurements conducted with other Cobas serology systems, which share characteristics with the Elecsys. This should make quantitative HBsAg diagnostics more easily accessible around the globe.

In summary, the current study shows a high correlation and agreement between quantitative HBsAg measurement conducted with the Architect and Elecsys assays, irrespective of HBV geno-

type. The performance of a clinical stopping rule established using Architect measurements was excellent when applied on Elecsys data. Both assays can therefore be applied for HBsAg quantification in clinical practice.

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Ethical approval

Obtained.

Disclosures

Milan J. Sonneveld, Charles A.B. Boucher, M.F.C. Beersma, L. Zwang and Bettina E. Hansen have nothing to disclose. Vincent Rijckborst is a consultant for Roche. H.L.A. Janssen received grants from and is a consultant for: Bristol Myers Squibb, Gilead Sciences, Novartis, Roche, and Merck/Schering Plough.

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