Journal of Clinical Virology 56 (2013) 306–311

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Quantification of hepatitis B e antigen between Elecsys HBeAg and Architect HBeAg assays among patients infected with hepatitis B virus

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ARTICLE INFO

Article history:
Received 23 August 2012
Received in revised form
28 November 2012
Accepted 13 December 2012

Keywords:
Hepatitis B e antigen
Hepatitis B virus
Human immunodeficiency virus
Architect
Elecsys

ABSTRACT

Background: Among patients infected with hepatitis B virus (HBV), quantification of hepatitis B e antigen (HBeAg) is accruing substantial clinical relevance as a marker for HBeAg-loss during treatment. No direct comparison has been made between assays that quantify HBeAg.
Objectives: To compare the performance of HBeAg quantification (qHBeAg) between Architect and Elecsys HBeAg assays among 183 patients with chronic HBV infection (94 treatment-naïve HBV-monoinfected and 89 antiretroviral-experienced HIV-HBV co-infected).
Study design: qHBeAg was determined in Paul Erlich Institute Units (PEIU)/mL using previously designed protocols. Values were compared with correlation and linear regression. Bland–Altman analysis was used to compare mean differences ($\bar{d}$) between Elecsys and Architect assays and limits of agreement (LOA) (±2 standard deviations [SD]).
Results: Between-assay correlation was significant overall ($r = 0.970$), yet stronger for qHBeAg < 1000 ($n = 131$) versus >1000 PEIU/mL ($n = 52$) as determined by the Elecsys assay ($r = 0.969$ vs. 0.880, respectively). On average, the Elecsys assay reported qHBeAg at 13.3 PEIU/mL lower than the Architect assay (LOA: −415.9, 389.3), while LOA between assays were much wider at higher levels (<1000: −198.2, 147.9; ≥1000 PEIU/mL: −688.4, 721.5). Further analysis indicated that $\bar{d}$ did not change substantially with respect to HBV genotype, *precore* mutation, and CD4+ cell count, regardless of HBeAg-level. Nevertheless, seven of eight patients with highly divergent between-assay results had HBV-DNA > 2000 IU/mL.
Conclusions: Elecsys and Architect assays report similar qHBeAg units with high correlation. Since qHBeAg was performed using an in-house approach, a commercially-available assay could reduce between-assay discrepancies, especially at higher HBeAg-levels.

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; qHBeAg, HBeAg quantification; HIV, human immunodeficiency virus; PEI, Paul Erlich Institute; $\bar{d}$, mean differences between Elecsys and Architect assays; LOA, limits of agreement; SD, standard deviation; NA, nucleos(t)ide analogs; CHB, chronic hepatitis B; TDF, tenofovir dipivoxil fumarate; PCR, polymerase chain reaction; BCP, basal core promoter; RLU, relative light units; PEI, Paul Ehrlich Institute; COI, cut-off index; CV%, coefficient of variation.

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http://dx.doi.org/10.1016/j.jcv.2012.12.022

1. Background

Chronic infection with hepatitis B virus (HBV) is a major cause of liver-related mortality among both mono-and human immunodeficiency virus (HIV) co-infected patients.[1] In patients with hepatitis B e antigen (HBeAg), HBeAg-seroconversion is major clinical endpoint indicating a long-lasting therapeutic response and clinical improvement.[2] Although HBV-DNA quantification is the most often used marker to determine treatment effectiveness,[3] rapid suppression of HBV-DNA viral load during treatment with nucleos(t)ide analogs (NA) cannot accurately predict HBeAg-seroconversion.[4]

Qualitative HBeAg assays have been used for several decades as a means to monitor patients infected with chronic hepatitis B (CHB). More recently, HBeAg quantification (qHBeAg) before and during treatment have been shown to predict response during pegylated interferon and/or HBV NA therapy,[5–13] while increasing HBeAg levels have been able to identify early viral breakthrough during lamivudine therapy.[7,11,14–16] Unfortunately, no commercial assay is available for qHBeAg, thereby stressing the need for high-performing, accurate, and reproducible assays.

2. Objectives

The aim of the present study was to compare performances between the Architect HBeAg and Elecsys HBeAg assays, specifically in HBV and HIV-HBV infected patients. Differences in qHBeAg between the two systems were also investigated with respect to a variety of HBV and, for co-infected patients, HIV co-factors.

3. Study design

3.1. Study population

A total of 183 chronically infected HBV-infected patients were included from two separate cohorts – one with 94 treatment-naïve HBV-monoinfected patients[17] and the other with 89 antiretroviral-experienced, HIV-HBV co-infected patients initiating treatment with tenofovir dipivoxil fumarate 300 mg per day as part of their combined antiretroviral therapy.[18] Patients were selected on the following criteria: HBsAg seropositivity for at least 6 months, HBeAg-positive serology, and an available serum sample. Patients with HIV-HBV co-infection (respectively compared to HBV monoinfection) were older (median age: 40 vs. 31 years), more predominately male (95.5% vs. 74.5%), more likely to come from a region of high HBsAg-seroprevalence (>8%: 57.5% vs. 6.7%), less likely to harbor HBV genotype B or C (0% vs. 39.7%), and had lower HBV-DNA replication (median $\log_{10}$ IU/mL: 4.29 vs. 7.25) ($p < 0.001$).

3.2. HBV quantification, genotype, and mutations

For HBV-monoinfected patients, serum HBV-DNA levels were measured using the Cobas® Ampliprep/Cobas TaqMan® assay (Roche Diagnostics, Mannheim, Germany). HBV genotyping and detection of *precore* and basal core promoter (BCP) mutations in the HBV genome were carried out by reverse hybridization using INNO-LiPA HBV and INNO-LiPA HBV precore assays (Innogenetics, Ghent, Belgium).

For HIV-HBV co-infected patients, serum HBV-DNA was quantified by a real-time polymerase chain reaction (PCR) assay (PCR-Amplicor; Roche Diagnostic Systems, Meylan, France). HBV genetic information was obtained on a subset of patients with HBV-DNA > 190 IU/mL. *Precore* nucleotide 1896, BCP dinucleotide 1762/1764, and clade genotyping were determined by DNA sequencing or DNA chip technology (bioMerieux, Marcy l'Etoile, France). Mutations in the YMDD motif (rt domain of the *pol* gene) were determined using PCR and direct sequencing.

3.3. HBeAg quantification assays

The Architect i2000 analyzer (Abbott Laboratories, Rungis, France), while using an automated chemiluminescent microparticle immunoassay, semi-quantitatively determines HBeAg interpreted as the ratio of sample relative light units (RLU) to cut-off RLU. Using the Modular E170 analyzer, the Elecsys HBeAg assay (Roche Diagnostics, Meylan, France) is an electrochemiluminescence immunoassay, where HBeAg is determined qualitatively and interpreted using the ratio of the sample signal to the cut-off signal. During a first incubation step, biotinylated monoclonal HBeAg-specific antibodies and monoclonal HBeAg-specific antibodies labeled with a ruthenium complex are added to the sample and form a sandwich complex with circulating HBeAg. During a second incubation step, streptavidin-labeled microparticles are added and the complex is bound to the solid phase via the biotin and streptavidin-interaction, resulting in an electrochemiluminescent signal. For these two assays, HBeAg concentrations were calculated with the method of Perrillo et al.[5] using the Paul Ehrlich Institute (PEI) standard.

In an initial step, a conversion factor for cut-off index (COI) and PEIU/mL needed to be established at various levels of qHBeAg. Using reference standards obtained from the PEI (Langen, Germany), a reference preparation with HBeAg concentration defined at 100 PEIU/mL (Reference 82) was initially diluted at 1:10 with PBS as a stock solution according to the manufacture's recommendations. Then, samples with HBeAg concentrations descending from 10 to 0.5 PEIU/mL were generated by 1:2 serial dilutions. HBeAg reference preparations were diluted twice-separately with two diluents for the Architect assay [the Architect Multi-Assay Manual and the HBsAg qualitative II Confirmatory Manual Diluents (Abbott)] and with one diluent for the Elecsys assay [using Universal Diluent as recommended, Roche Ref: 11732277 122)]. Each standard concentration unit was compared to the assay signal and a conversion factor was calculated. The Architect assay was tested in triplicate, whereas one measure was used for the Elecsys assay since previous evaluations have demonstrated low coefficient of variation (CV%).[16]

qHBeAg was performed retrospectively on patient sera stored at −80 °C and performed in two batches (the first being among HIV-HBV co-infected patients and the second HBV-monoinfected patients). For both assays, patient samples were initially tested undiluted. In order to avoid any hook effect in sera with high levels of circulating HBeAg, samples with levels >100 COI were then diluted at 1:40.

3.4. Statistical analysis

Assay precision was given for all replicated measures by the mean, standard deviation (SD), and CV% at each HBeAg concentration.

Values from both methods were plotted and compared using Pearson's correlation. We first performed a simple linear regression model, stratified on batch, using Elecsys assay as the dependant variable and the Architect assay as the independent variable. The coefficient between measures, which should be approximately equal to 1.0, was higher than anticipated (Supplementary Fig. 1). We then performed a Bland–Altman analysis in which the pair difference between Elecsys and Architect methods were plotted against the pair mean, showing a clearly linear relationship for both batches (Supplementary Fig. 2). As a result, we modeled the residuals of the between-assay difference (individual differences subtracted by the population mean difference) across the