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Article type : Original Article

**Clinical utility of quantifying hepatitis B surface antigen in African patients with chronic hepatitis B**

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**Key words:** Chronic Hepatitis B, Hepatitis B Surface Antigens, Africa, Selection for Treatment, Serologic Tests
**Word account:** 2,794
**References:** 31
**Tables:** 2
**Figures:** 2
**Suppl. Tables:** 2

**Abstract (n=250 words)**

The clinical utility of quantifying hepatitis B surface antigen (qHBsAg) levels in African subjects with chronic hepatitis B virus (HBV) infection has been poorly documented.From a multicenter cohort of 944 HBV-infected African patients we aimed to assess whether qHBsAg alone can accurately identify i) those in a HBeAg-negative chronic HBV infection phase at low risk of liver disease progression and ii) those in need of antiviral therapy according to the 2017 EASL guidelines.We analyzed 770 HBV mono-infected treatment-naïve patients, mainly males (61%) from West Africa (92%), median age 35 years (IQR: 30-44), median HBV DNA: 95.6 IU/ml (10.0-1,300.0), median qHBsAg 5,498 IU/ml (1,171-13,000), HBeAg-pos 38 (5%). A total of 464/770 (60.2%) patients were classified as HBeAg-negative chronic infection (median age 36 years (31-46), median ALT 23 IU/l (18-28), median HBV-DNA 33.5 IU/ml (3.8-154.1), median LSM 4.8 kPa (4.1-5.8)) and qHBsAg levels had poor accuracy to identify these subjects with an AUROC at 0.58 (95%CI: 0.54-0.62), sensitivity 55.0%, specificity 55.6%; 118/770 (15.3%) patients were eligible for treatment according to the 2017 EASL criteria. qHBsAg correlated poorly with HBV DNA and had poor accuracy to select patients for antiviral therapy with an AUROC at 0.54 (0.49–0.60), sensitivity 46.6%, specificity, 46.9%.In African treatment-naïve HBV-infected subjects, the clinical utility of qHBsAg to identify subjects in HBeAg-negative infection phase or subjects eligible for antiviral therapy seems futile. Whether qHBsAg levels can be used as a predictor of long-term liver complications in Africa needs to be further investigated.

Infection with hepatitis B virus (HBV) is a major public health issue worldwide. Sub-Saharan Africa accounts for an estimated 60 million people chronically infected with HBV (1). However, access to HBV diagnosis and treatment is limited in most African countries with only 2% of HBV-infected people diagnosed and less than 1% of those eligible for antiviral therapy receiving treatment in 2016 in sub-Saharan Africa (2). In patients with chronic HBV infection, the assessment of liver disease and initiation of antiviral therapy mainly rely on three measurements: alanine aminotransferase (ALT) levels, HBV viral load, and liver fibrosis estimation using liver histology or liver stiffness measurement (e.g. by vibration-controlled transient elastography [Fibroscan®]) (3). Except for ALT levels, these tests are difficult to perform in clinical practice in Africa and are therefore major barriers to scale-up HBV screen-and-treat interventions in this region. Indeed, measurement of HBV viral load currently relies on nucleic acid testing using quantitative real-time polymerase chain reaction (qRT-PCR), which is expensive (up to €150 in Africa) and requires high quality laboratories and well-trained technicians. In addition, Fibroscan® devices remain expensive and are often only accessible in capital cities in private sectors (4). To overcome these limitations, simplified diagnostic tools and algorithms are needed. For liver fibrosis assessment, biochemical scores (e.g aspartate-aminotransferase (AST)-to- -platelet ratio index (APRI) or gamma glutamyl-transpeptidase to platelet ratio (GPR)) have been proposed but their diagnostic accuracy is debated in African populations (5, 6). To quantify HBV viral replication, new molecular technologies (e.g GeneXpert) (7) or serological biomarkers (e.g Hepatitis B core-related antigen (HBcrAg)) (8) have been validated as accurate alternatives to the conventional qPCR, but they are still difficult to implement at large scale in resource-limited countries.

HBV-infected subjects in the HBeAg-negative chronic infection phase (previously known as chronic inactive carriers) should be at low risk of liver disease progression (9). Therefore, in Africa, where a large proportion of HBV-infected people are in this phase (10) a simple biomarker to easily identify these subjects and those in need of antiviral therapy would be extremely useful to scale-up HBV screen-and-treat interventions.

In the last decade the quantification of serum HBsAg (qHBsAg) levels, which reflects the transcriptional activity of covalently closed circular DNA (cccDNA), has been used routinely in high-income countries, to define the phase of the infection and predict liver-related complications in patients with chronic HBV infection (11, 12). Levels of qHBsAg have been also identified as a useful marker to monitor response to treatment and predict relapse after stopping nucleos(t)ide analogue therapy (13). Although qHBsAg levels is not recommended to select patients for antiviral therapy, its clinical utility to predict or monitor response to treatment is mentioned in international HBV guidelines (3). HBsAg quantification is a simple and more affordable test (less than €2 per test) than qPCR.

Data on qHBsAg levels in HBV-infected patients has been mainly collected in Western (13-15) and Asian countries (16-19). In African HBV-infected subjects, qHBsAg levels and its clinical utility have been very inadequately documented (20, 21) with conflicting results. In particular, whether it could simplify the identification of subjects in an HBeAg-negative chronic infection phase and of patients in need of antiviral treatment in Africa is unknown.

We hypothesized that qHBsAg levels could be used in clinical routine to easily distinguish patients in need of antiviral therapy from those in an HBeAg-negative chronic infection phase considered at low risk of liver disease complications.

This study aimed to assess whether HBsAg levels alone is accurate to 1) identify subjects in a HBeAg-negative chronic HBV infection phase and 2) select subjects for antiviral therapy amongst treatment-naïve HBV-infected people in Africa.

## Methods

### Study population

Data from treatment-naïve, HBV-infected African patients, enrolled in African (Burkina Faso/The Gambia/Senegal) and European (France/Germany) cohorts, were retrospectively analyzed. Data included basic demographic information (age, gender, excessive alcohol intake, country of birth), as well as laboratory data (HBV viral load, qHBsAg levels, Hepatitis B e antigen (HBeAg) serology (ETI-EBK Plus, Diasorin, Italy or Abbott Diagnotics, Chicago, IL, USA), liver enzyme levels (ALT, AST, GGT, platelet count, and co-infection sero-status (HIV), hepatitis C virus (HCV), hepatitis Delta virus (HDV)). Patients with excessive alcohol intake as defined by intake >20g/day or HIV, HCV or HDV co-infections were excluded from the final analysis.

### Laboratory investigations

### HBV DNA measurement

HBV DNA levels were measured by qPCR using commercialized assay (Abbott Diagnotics, Chicago, IL, USA) in all countries except The Gambia where a validated in-house qPCR was used (22).

### HBs antigen quantification

qHBsAg levels were measured in all patients using the Abbott Architect chemiluminescent microparticule assay (Abbott Diagnotics, Chicago, IL, USA). Samples were tested at dilution 1/500 as recommended with a range of quantification of 25-125,000 IU/ml after dilution. We also calculated the qHBsAg/HBV DNA ratio that reflects the relationship between HBsAg production and HBV replication and might be a better marker of viral activity than HBV viral load or qHBsAg levels alone (23).

### HBV genotyping

HBV genotyping was determined in a subgroup of patients. PCR and nested PCR products were sent to Genome Express (Grenoble, France) for sequencing. Edited sequences were submitted to BLAST analysis, and the highest scoring complete HBV genome was retained. A phylogenetic tree was built from >2000 complete HBV genome sequences contained in GenBank, and HBV genotype/subgenotype clades were identified based on information in the GenBank entries and/or in original publications.

### Liver fibrosis assessment

The severity of liver fibrosis was assessed using fasting LSM in all patients. As recommended, a valid Fibroscan® (Echosens, France) value was defined as at least 10 valid measurements, a success rate of at least 60% and an IQR/median-ratio of less than 30% (24). To stage the degree of liver fibrosis, we used previously validated LSM cut-offs in African patients with chronic HBV infection: ≥7.9 kPa for clinically significant fibrosis (≥F2) and ≥9.5 kPa for cirrhosis (6). In 88 patients liver biopsies were carried out and fibrosis grading was reported in METAVIR score.

### HBV infection phases

We determined the phase of HBV infection according to the EASL 2017 guidelines using a single time point analysis (supplemental table 1). Subjects in the HBeAg-negative HBV infection phase were defined as having a negative HBeAg serology and HBV DNA <2.000 IU/ml and ALT<40 IU/L and none or mild liver fibrosis based on LSM (<7.9kPa) or liver histology analysis if available (F0-F1) and no or mild activity (A0-A1).

### Hepatitis B treatment eligibility

We applied the 2017 EASL treatment criteria based on a single time point as usually done in resource-limited areas. We applied the upper limit of normal for ALT as 40 IU/L irrespective of gender as recommended by EASL (3) (Suppl. Table 2).

### Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 24. We report means and standard deviation for all metric and normally distributed variables. If normal distribution could not be assumed, median and interquartile ranges (IQR) are presented. Since none of our variables shows a normal distribution, we used Mann-Whitney-U and Kruskal Wallis tests as well as Spearman's correlation coefficient to compare or correlate different variables. A two-sided P value of less than 0.05 was considered statistically significant.

The capability of qHBsAg levels to correctly identify patients with HBeAg-negative chronic infection, as well as to select patients for antiviral treatment, were evaluated by the receiver operating characteristic (ROC) curve. The optimal cut-offs for HBsAg levels were selected to minimize the absolute difference between the sensitivity and specificity.

## Results

### Study population

We extracted data from 944 treatment-naïve African patients with chronic HBV infection and available qHBsAg levels. Most of them were recruited in West Africa (n=882) through the PROLIFICA research program (10, 25) in The Gambia and Senegal (n=689). A minority of African patients (n=149) was enrolled in European cohorts. Finally, complete data from 770 treatment-naïve, HBV mono-infected African patients were analyzed (Figure 1). Table 1 summarizes the characteristics of the study population according to the different phase of HBV infection.

All patients were born in Africa and were mainly from West Africa (n=711, 92.3%), a minority of patients was from North Africa (n=14, 1.8%) and Central or East Africa (n=45, 5.8%). Most of them were males (n=468, 61%), median age of 35 years (IQR: 30-44), 38 (4.9%) subjects were tested positive for HBeAg, median LSM was 5.3 kPa (4.4 – 7.0) with 133 patients having significant liver fibrosis (7.9%) or cirrhosis (9.4%), median ALT level was 25 IU/L (20 - 36) and median HBV DNA level was 95.6 IU/ml (10.0–1,300.00) with a median qHBsAg levels of 5,497.6 IU/ml (1,170.8 – 13,000.0). HBV genotype was determined in a subgroup of 202 patients: Genotype E was predominant (178 (88.1%)) whilst a minority of patients were infected with genotype A (24 (11.9%)).

The HBsAg levels were higher in HBeAg-positive patients compared to HBeAg-negative patients (10,407.5 IU/ml (2,582.5 – 33,320.0) vs 5,343.4 IU/ml (1,142.1 – 13,000.0), p=0.003). HBsAg levels were higher in patients classified with HBeAg-negative chronic hepatitis than in those with HBeAg-negative chronic infection (7,025.39 IU/ml (2,305.71 – 14,694.00) vs 4,526.1 IU/ml (460.2 – 13,000.0), p<0.001) (Table 1).

qHBsAg to HBV DNA ratio also differed according to HBeAg sero-status (0.01 (0.00 – 0.18) vs. 17.3 (0.6 – 268.0, p<0.001). HBV DNA ratio was the highest in patients with HBeAg-negative chronic infection 60.8 (4.1 – 561.3) and higher than that observed in HBeAg-negative chronic hepatitis subjects 1.37 (0.14 - 24.72), p<0.001.

### Correlation between HBsAg levels and HBV viral load

In the whole study population, including both HBeAg-positive and HBeAg-negative subjects, there was a poor correlation between qHBsAg levels and HBV DNA levels (r=0.270, p<0.001). In a subgroup of HBeAg-positive patients the correlation was higher as compared to the correlation observed in HBeAg-negative patients (r=0.565 and r=0.252, p<0.001 respectively) (Figure 2). In a subset of patients with available HBV genotype determination (n=202), the correlation of qHBsAg with HBV DNA was poor even after stratifying by the genotypes (r=0.090, p=0.234 in genotype E and r=-0.043, p=0.843 in genotype A).

**Performance of qHBsAg to identify patients with HBeAg-negative chronic infection.**

In the entire study population 464 (60.3%) subjects were identified as having HBeAg-negative chronic infection (Table 1). The performance of qHBsAg levels was poor to identify these subjects with an AUROC at 0.58 (0.54 – 0.62), sensitivity 55.0%, specificity 55.6% for a best-identified threshold of 5,660 IU/ml (Table 2).

**Performance of qHBsAg levels to predict HBV treatment eligibility**

Applying the 2017 EASL guidelines, 118 (15.3%) were eligible for antiviral therapy. Using the 2017 EASL criteria as a reference, qHBsAg levels failed to correctly identify patients eligible for treatment with an AUROC at 0.54 (0.49 – 0.60) and low sensitivity and specificity at 46.6% and 46.9% respectively, with a best identified cut-off of 6,000 IU/ml (Table 2).

Our results did not differ between patients living in Africa and those living in Europe and were similar irrespective of the use of commercial or in-house HBV PCR (data not shown).

## Discussion

In a large cohort of African treatment-naïve patients with chronic HBV infection, we found that qHBsAg levels had a poor clinical utility to identify HBV-infected subjects with HBeAg-negative chronic HBV infection or to select HBV-infected subjects for antiviral therapy in Africa.

In most African countries, hepatitis B screening interventions are usually based on only the detection of HBsAg using a rapid POC test after finger prick, and people have a significantly limited access to HBV DNA measurement. We could not confirm our hypothesis that qHBsAg levels could facilitate a rapid triage of HBV-infected patients.

To the best of our knowledge the utility of qHBsAg has never been examined in HBV-infected African patients, so far. To date, only two studies analyzed the levels of qHBsAg in African subjects; one study from Senegal assessed the fluctuation of qHBsAg levels in 87 HBV-infected patients with normal ALT level (20) but did not examine whether qHBsAg levels could help classifying the phase of natural history of chronic HBV infection; another study conducted in the UK assessed the qHBsAg levels in 259 Genotype E patients born in West Africa, but only included patients with a viral load above 2,000 IU/ml and raised ALT level (21).

As previously shown in Asian (19) and European (15) patients, our study found that qHBsAg levels varied according to the positivity of HBeAg in African HBV-infected patients with the lowest levels observed in HBeAg-negative patients and the highest levels measured in HBeAg-positive patients. We also found that qHBsAg to HBV DNA ratio was the highest in subjects with HBeAg-negative chronic infection as previously suggested (14, 26). In this phase of the infection, HBsAg is transcribed from integrated DNA while cccDNA transcription is inhibited in this phase (26-29).

As observed in other studies (19-21, 29), we found a poor correlation between qHBsAg levels and HBV viral load, especially in HBeAg-negative subjects. In West African HBV-infected patients with viral load ≥2,000 IU/ml and raised ALT level, Chakrabarty et al. found lower qHBsAg levels in patients with advanced liver fibrosis (21). We also found low level of HBsAg in subjects with cirrhosis but no correlation was observed between LSM and qHBsAg levels in our study (data not shown).

We assessed the accuracy of qHBsAg levels for the identification of HBeAg-negative subjects with chronic infection. From a public health perspective, the validation of a simple marker to easily identify patients in this phase is highly needed in Africa. Indeed, the vast majority of HBV-infected subjects in Africa are classified in this phase (10) and might be at low risk of liver disease progression (9) suggesting a possible cost-effective one-step liver assessment with major cost savings for resource-limited African countries. Unfortunately, in contrast to previous findings (14, 30), qHBsAg levels failed in our study to accurately identify subjects in this phase. This could be explained by relatively high levels of qHBsAg levels of subjects with HBeAg-negative chronic infection in our study (median 4526.1 IU/ml (460.2 – 13,000) despite very low median HBV viral load 33.50 IU/ml (3.79 - 154.07)) as reported in previous African studies (20, 21). Asian and Western studies (14, 16) reported much lower qHBsAg levels in their population of inactive chronic carriers. Indeed, qHBsAg levels do not reflect the production of virions but rather the presence of defective HBsAg particles (spheres and filaments) that can dramatically exceed the amount of competent infectious virions by $10^2$ to $10^5$. Whether the high levels of qHBsAg observed in African subjects in the HBeAg-negative chronic infection phase is attributable to genotypes or genetic variability of HBV, or clinical outcomes in Africa, remains to be confirmed.

Using the 2017 EASL treatment criteria, we also confirmed the poor performance of qHBsAg levels for identifying subjects in need of antiviral therapy. From a public health perspective the identification of patients in immediate need of antiviral therapy based on a one-step screening intervention would be very useful in resource-constrained areas in Africa.

Our study has some limitations. First, patients were assessed on a single time point; however, most patients with HBV infection living in resource-limited countries have a single assessment; we were unable to provide longitudinal data at this stage. Therefore, we did not assess the

| | Full study population n=770 | HBeAg- CI n=464 | HBeAg- CH N=206 | HBeAg+ CI n=13 | HBeAg+ CH n=15 | Cirrhosis n=72 | P value* |
|---|---|---|---|---|---|---|---|
| **Age, years** | 35 (30 - 44) | 36 (31 - 46) | 35 (29 - 40) | 32 (28 - 38) | 32 (28 – 37) | 36 (30 – 42) | P=0.004 |
| **Males, n** | 468 (60.8%) | 238 (51.3 %) | 148 (71.8 %) | 8 (61.5 %) | 12 (80.0 %) | 62 (86.1 %) | p<0.001 |
| **West African, n** | 711 (92.3%) | 448 (96.6 %) | 169 (82 %) | 12 (92.3 %) | 10 (66.7 %) | 72 (100 %) | p<0.001 |
| **East African, n** | 45 (5.8 %) | 14 (3.0 %) | 29 (14.1 %) | 1 (7.7 %) | 1 (6.7 %) | 0 | p<0.001 |
| **North African, n** | 14 (1.8 %) | 2 (0.4 %) | 8 (3.9 %) | 0 | 4 (26.7 %) | 0 | p<0.001 |



utility of qHBsAg levels to predict the development of liver disease complications over time. We will however address this question in the future. Secondly, our study population was mainly from West Africa and therefore the proportion of non-E genotype was small. Thirdly, we used two different methods for HBV DNA quantification; however, our findings were similar irrespective of the use of a commercial or in-house PCR. Fourthly, we did not analyze the impact of pre-core and pre-S mutations on the qHBsAg levels although these mutations may influence the level of HBsAg and contribute to liver disease complications (17, 31)

In conclusion, although the quantification of HBsAg levels is a simple and inexpensive test its clinical utility to identify subjects at low risk of liver disease progression (HBeAg-negative chronic infection phase) or subjects eligible for immediate antiviral treatment in Africa is poor. Additional markers and strategies are needed to simplify the stratification of HBV-infected subjects in order to scale up screen-and-treat interventions in Africa.

**Authors ‘contribution:** ML,PI,GP,YS,JH,MT designed the study. GP,ML,YS were in charge of the statistical analysis. AS,JH,AC,GL,IC,CTK were in charge of the virological analysis. ML,GP,PI,YS drafted the first manuscript. All authors contributed to patient recruitment and approved the manuscript.

**Conflicts of interest:** ML, PI, YS, MT received research funding from Gilead US.

**Funding:** the PROLIFICA project (www.prolifica.africa) was funded was the EC FP7 (2011-2016) and is currently funded by the MRC UK and received support from Gilead US company. The authors thank Echosens, France for their support; they are also grateful to all the patients and their families.

**Data availability statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Tables:**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **LSM, kPa** | 5.3 (4.4 – 7.0) | 4.8 (4.1 – 5.8) | 5.9 (4.8 – 8.0) | 5.3 (3.3 - 6.5) | 6.6 (5.9 - 7.8) | 12.45 (10.7 - 17.6) | p<0.001 |
| **HBV DNA (IU/ml)** | 95.64 (9.98 – 1300.00) | 33.50 (3.79 - 154.07) | 2781.11 (110.06 - 20804.00) | 7801425.18 (330451.31 – 129130845.8) | 632.4 (4.29 - 584370.55) | 2124.03 (164.57 – 69870.67) | p<0.001 |
| **qHBsAg (IU/ml)** | 5497.62 (1170.77 – 13000.0) | 4526.1 (460.2 – 13000) | 7025.39 (2305.71 – 14694.00) | 33320.00 (13196.00 – 52000.00) | 7771.00 (574.30 – 27267.28) | 4720.8 (1204.94 – 7839.58) | p<0.001 |
| **qHBsAg to HBV DNA ratio** | 13.26 (0.36 – 238.81) | 60.8 (4.1 – 561.3) | 1.37 (0.14 - 24.72) | 0 (0 - 0.01) | 0.21 (0.01 - 22.36) | 1.09 (0.04 – 10.67) | p<0.001 |
| **HBV Genotype, n, m=202** | | | | | | | |
| **E** | 178 (88.1 %) | 106 (89.8 %) | 41 (85.4 %) | 5 (83.3 %) | 3 (100 %) | 23 (85.2 %) | p=0.186 |
| **A** | 24 (11.9 %) | 12 (10.2 %) | 7 (14.6%) | 1 (16.7 %) | 0 | 4 (14.8 %) | p=0.511 |
| **ALT, IU/l** | 25 (20 – 36) | 23 (18 - 28) | 43 (25 - 58) | 32 (24 – 33) | 46 (38 – 67) | 35 (25 - 57) | p<0.001 |
| **AST, IU/l, m=765** | 30 (25 - 37) | 27 (23 - 32) | 37 (29 - 48) | 34 (28 – 39) | 40 (34 – 55) | 37 (31 – 65) | p<0.001 |
| **GGT, IU/l, m=723** | 26 (20 - 38) | 24 (18 - 31) | 33 (24 - 49) | 34 (20 – 46) | 28 (19 – 56) | 38 (26 – 72) | p<0.001 |
| **Platelet count, $10^9$/L , m=731** | 205(163 – 251) | 212 (171 - 257) | 194 (158 -246) | 243 (195 – 273) | 190 (137 – 235) | 147 (123 – 209) | p<0.001 |
| **2017 EASL treatment eligibility, n** | 118 (15.3 %) | 5 (1.1 %)† | 29 (14.1 %) | 8 (61.5 %) | 4 (26.7 %) | 72 (100%) | p<0.001 |

**Table 1.** Characteristics of the study population according to the HBV infection phases. *based on either Kruskal Wallis or Chi-squared test. Data are presented as median (IQR) or n (%). † all 5 patients had a family history of HCC.

HBeAg- CI = HBeAg negative chronic infection, HBeAg- CH = HBeAg negative chronic hepatitis, HBeAg+ CI = HBeAg positive chronic infection, HBeAg+ CH = HBeAg positive chronic hepatitis, LSM = liver stiffness measurement, HBV DNA = Hepatitis B virus deoxyribonucleic acid, qHBsAg = quantification of serum HBsAg, ALT = alanine aminotransferase, AST= aspartate aminotransferase, GGT = gamma-glutamyl transferase.

| | Entire study population n=770 | |
|---|---|---|
| | **qHBsAg level (IU/ml) to identify HBeAg-neg CI subjects** | **qHBsAg level (IU/ml) to select patients to antiviral therapy** |
| **AUROC (95% CI)** | 0.58 (0.54 - 0-62) | 0.54 (0.49 - 0.60) |
| **Optimal cut-off** | 5,660 | 6,000 |
| **Sensitivity (%)** | 55.0 | 46.6 |
| **Specificity (%)** | 55.6 | 46.9 |
| **PLR** | 1.24 | 0.88 |
| **NLR** | 0.81 | 1.14 |

**Table 2:** Performance of HBsAg levels for the identification of HBeAg-neg chronic infection (CI) patients (left column) and treatment eligibility (right column)

Offs: *Abbreviations: AUROC: area under the receiver operating characteristics, CI: chronic infection, PLR: positive likehood ratio, NLR: negative likehood ratio*

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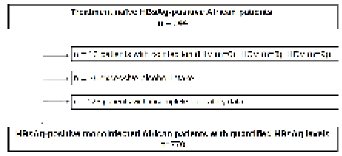
**Figure legends**

**Figure 1:** Study flow chart

**Figure 2**: Correlation between HBsAg levels [IU/ml] and HBV viral load [IU/ml] in the whole study population (A), in HBeAg-negative patients (B) and HBeAg-positive patients (C). Axis are on logarithmic scale.

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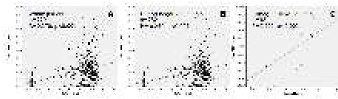

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