

The contribution of more sensitive hepatitis B surface antigen assays to detecting and monitoring hepatitis B infection

Charlotte Pronier^a, Daniel Candotti^b, Laure Boizeau^b, Jérémy Bomo^a, Syria Laperche^b, Vincent Thibault^{a,*}

^a Univ Rennes, Department of Virology, CHU Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085, F-35000, Rennes, France

^b Department of Blood-borne Agents, National Reference Center of Infectious Risks in Blood Transfusion, Institut National de la Transfusion Sanguine, Paris, France



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ABSTRACT

Background: Hepatitis B surface antigen (HBsAg) remains the main viral marker for screening and monitoring hepatitis B virus (HBV) infection. The quantification limit of most current HBsAg assays is around 0.05 IU/mL. The Lumipulse-G-HBsAg-Quant assay (Fujirebio) claims to obtain a tenfold improvement in sensitivity. This study aimed to assess the performance of this assay in detecting low HBsAg levels in clinical samples.

Methods: Three panels of stored frozen samples were selected on the basis of HBV-DNA and HBsAg values obtained previously with routine techniques. Panels 1 (n = 13) and 2 (n = 52) consisted of DNA-positive/HBsAg-negative samples from individuals in the window period and with occult HBV infection respectively. Panel 3 comprised 23 samples with low or discrepant HBsAg screening results. All these samples were tested retrospectively with the DiaSorin and Fujirebio HBsAg assays.

Results: Sixteen out of 65 samples (25 %), initially screened HBsAg negative, were reactive only with the Fujirebio assay (median value = 0.015 IU/mL; IQR = 0.012): three (23 %) samples from panel 1 and 13 (25 %) from panel 2. Thirteen of these 16 (81 %) had HBsAg values below 0.03 IU/mL with the DiaSorin assay. In panel 3, 22 (96 %) samples were quantified successfully with the Fujirebio assay (median: 0.32 IU/mL; IQR: 1.20) and 19 (83 %) with the DiaSorin assay (median: 0.31 IU/mL; IQR: 0.65). Concentrations obtained with the two assays showed good correlations ($r = 0.893$, Spearman).

Conclusions: HBsAg assays with enhanced analytical sensitivity could improve HBV serological profile interpretation with possible consequences on clinical management of infected patients, and on blood transfusion safety.

1. Introduction

Despite a vaccine to prevent Hepatitis B virus (HBV) infection and antivirals to control replication, hepatitis B remains a public health problem and one of the leading causes of chronic liver disease [1]. Due to the often silent nature of the disease, HBV testing is essential for individuals, public health, and blood screening.

Hepatitis B surface antigen (HBsAg), widely produced at all stages of infection, remains a key marker for the diagnosis and the monitoring of HBV infection. Following the incubation period, HBsAg often accompanied by anti-HBc IgM, can be detected a few days after HBV-DNA [2]. The greater the sensitivity of HBsAg detection, the sooner an acute infection can be identified through HBsAg screening. HBsAg assay sensitivity is a key issue, firstly in transfusion settings, in countries that

cannot afford to implement nucleic acid testing (NAT) of blood donations and rely solely on HBsAg testing. Increased HBsAg assay sensitivity could translate into an improved blood safety as it would reduce the pre-HBsAg window period (WP), classically defined as the time between infection and the appearance of HBsAg in the blood of infected persons [3]. This delay is currently established at 33.5 days but obviously depends on the HBsAg detection sensitivity. Secondly, HBV infection monitoring could benefit from improved sensitivity. DNA and HBsAg concentrations, with liver function and histology assessments are helpful to stage the infection evolution [4,5]. While HBsAg levels are usually high during the early chronic phases (HBeAg-positive), a concentration below 3 log IU/mL with low viral replication in HBeAg negative patients, usually signs an inactive form of infection. [6]. In a few cases, the disappearance of HBsAg indicates a "functional cure" [7].

* Corresponding author.

E-mail address: vincent.thibault@chu-rennes.fr (V. Thibault).

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Because small amounts of HBsAg can still be generated from intrahepatic HBV-DNA, improved HBsAg detection sensitivity could change a "cured" profile to a low-HBsAg level chronic carrier profile. Occult HBV infection (OBI), defined as the presence of replication-competent HBV-DNA in the liver and/or in the blood of people who test negative for HBsAg using currently available assays, would also be reappraised by more sensitive HBsAg assays [8]. Indeed, the failure to detect HBsAg despite viral replication could be explained by different hypotheses, one of them being that amounts of HBsAg are too small to be detected by current HBsAg assays. The identification of patients with low residual production of HBsAg could also be beneficial among immunosuppressed patients to better identify those at risk of reactivation [9,10].

The analytical characteristics of HBsAg testing have greatly improved over the years. Detection of HBsAg in serum is commonly carried out using enzyme immunoassays (EIA) based on chemiluminescence and polyclonal antibodies with exquisite sensitivity. An evaluation of 70 HBsAg assays showed unequal performances in term of sensitivity between EIAs and rapid tests, but also among EIAs [11]. The coexistence of HBsAg and anti-HBs in serum is identified in 5–10% of chronic hepatitis B carriers and could also constitute a challenge for HBsAg detection assays [12]. Ideally, the presence of anti-HBs should not influence HBsAg quantification. A new Japanese iTACT-HBsAg assay, which includes a pretreatment step, seems to detect HBsAg with greater sensitivity, including that complexed with anti-HBs [13]. This approach should certainly be considered for further development. HBV protein variability also presents a challenge for immunoassays, as they need to detect HBsAg variants for accurate diagnosis or for the prevention of transfusion-transmitted infection [14]. HBsAg assays marketed in Europe are CE-marked devices and should meet the requirements for sensitivity and specificity set out in common technical specifications. The analytical sensitivity of HBsAg assays has been fixed at 0.130 IU/mL (second international standard for HBsAg, subtype adw2, genotype A, NIBSC code 00/588). The lower limit of quantification (LoQ) for most current HBsAg quantification assays is around 0.05 IU/mL. Some assays such as the Lumipulse G HBsAg-Quant (Fujirebio, Tokyo, Japan) or the more recently reported ultra-sensitive Abbott ARCHITECT® assay have been developed and claim tenfold sensitivity improvement (LoQ 0.005 IU/mL) [15].

This study aimed to assess the performance of the highly sensitive Fujirebio assay in detecting low HBsAg levels in clinical samples and to evaluate the possible practical consequences of this increased sensitivity.

2. Material and methods

A total of 88 samples were collected from the INTS (national blood transfusion institute) and Rennes University Hospital. Plasma or serum samples were selected among individuals with documented HBV replication despite an undetectable HBsAg (n = 65): 13 samples were collected during the WP (panel 1) and 52 from occult B infection carriers (OBI; panel 2). A third panel (panel 3) comprised 23 blood donor samples from the INTS: 4 samples tested initially positive with the PRISM HBsAg 5.0 assay (Abbott Diagnostics, Rungis, France) at the time of donation but negative (S/CO: 0.70–0.88) when repeat-tested using the Murex HBsAg Version 3 assay (Diasorin, Antony, France); 18 samples with repeatedly detected but low HBsAg levels (median: 0.1 IU/mL [range: 0.07–1.21 IU/mL]); and 1 sample from a HBsAg + donor infected with a viral strain carrying substitutions in the S protein (sM103I, sS113A, sP120 T) and a stop codon at position 216.

Frozen samples were retrospectively tested after thorough thawing and mixing, according to the manufacturer's instructions with first the LIAISON® XL MUREX HBsAg Quant (DiaSorin, Antony, France) assay and then with the Lumipulse® G HBsAg-Quant (Fujirebio, Tokyo, Japan) assay. The dynamic ranges of the Fujirebio and DiaSorin assays announced were 0.005–150 IU/mL and 0.05–150 IU/mL, respectively.

Following Fujirebio's instructions (all details regarding samples processing are provided in the supplementary file) samples with an HBsAg value below 0.005 IU/mL with their assay were considered non-reactive. In our study, samples with an initial HBsAg titer between 0.002 and 1 IU/mL with the Fujirebio assay were tested twice to ascertain specificity of the result.

2.1. Complementary laboratory testing

On INTS samples, HBV-DNA was quantified using the COBAS TaqMan HBV assay (Roche Diagnostics; Meylan, France; LoQ ≥ 6 IU/mL). HBsAg, anti-HBc and anti-HBs antibodies were detected using Murex HBsAg Version 3 (Diasorin, Antony, France), Monolisa Anti-HBc PLUS (Bio-Rad, Marne-la-Coquette, France), and Monolisa Anti-HBs PLUS (Bio-Rad) respectively. HBV genotypes were determined by direct sequencing as previously described [16].

On Rennes University Hospital samples, the HBV-DNA level was measured using Realtime HBV (Abbott molecular, Rungis, France) or VERIS HBV (Beckman Coulter, Maurens-Scopont, France) assays with a LoQ ≥ 10 IU/mL. HBsAg, anti-HBc and anti-HBs antibodies were detected using Elecsys HBsAg, anti-HBc and anti-HBs II assays respectively (Roche Diagnostics, Meylan, France).

2.2. Statistical analysis

The HBsAg positive rates were compared using the McNemar test. Correlations and differences were analyzed using Spearman's coefficient and Bland-Altman plots. These analyses were performed using Analyse-it 4.65.3 statistical software package. P-values below 0.05 were considered statistically significant.

3. Results

The virological characteristics of the samples selected are presented in Table 1. HBV genotype information was available for 65 samples (73.9%). The most prevalent genotypes (53%) were A or D.

In all, 88 samples were tested with the DiaSorin and Fujirebio assays, as shown in Fig. 1. Only 19 (22%) samples were positive with DiaSorin, all belonging to panel 3 with HBsAg concentrations ranging

Table 1
Characteristics of the samples studied.

Number (%)	Total n = 88	WP n = 13	OBI n = 52	Low/ Discordant n = 23
HBV Genotype	65 (73.9)	13 (100)	41 (78.8)	11 (47.8)
■ A	25 (28.4)	5 (38.5)	17 (41.5)	3 (27.3)
■ B	6 (6.8)	1 (7.7)	3 (7.3)	2 (18.2)
■ C	3 (3.4)	0	2 (4.9)	1 (9.1)
■ D	22 (25)	3 (23.1)	15 (36.6)	4 (36.4)
■ E	7 (8)	3 (23.1)	4 (9.8)	0
■ F	2 (2.3)	1 (7.7)	0	1 (9.1)
HBV-DNA				
Not detected	3 (3.4)	0	0	3 (13)
Detected < 10 IU/mL	42 (47.7)	3 (23.1)	29 (55.8)	10 (43.5)
Detected ≥ 10 IU/mL	43 (48.9)	10 (76.9)	23 (44.2)	10 (43.5)
(median; IQR)	(106; 428)	(41; 229)	(106; 244)	(2042; 21,073)
Anti-HBc positive	75 (85.2)	0	52 (100)	17 (73.9)
Anti-HBs				
positive	27 (30.7)	3 (23.1)	18 (34.6)	6 (26.1)
concentration IU/L [min-max]	[11–414]	[193–414]	[11–227]	[11–95]
negative	58 (65.9)	10 (11.4)	31 (35.2)	17 (19.3)
not determined	3 (3.4)	0	3 (3.4)	0

WP: window period; OBI: occult HBV infection.

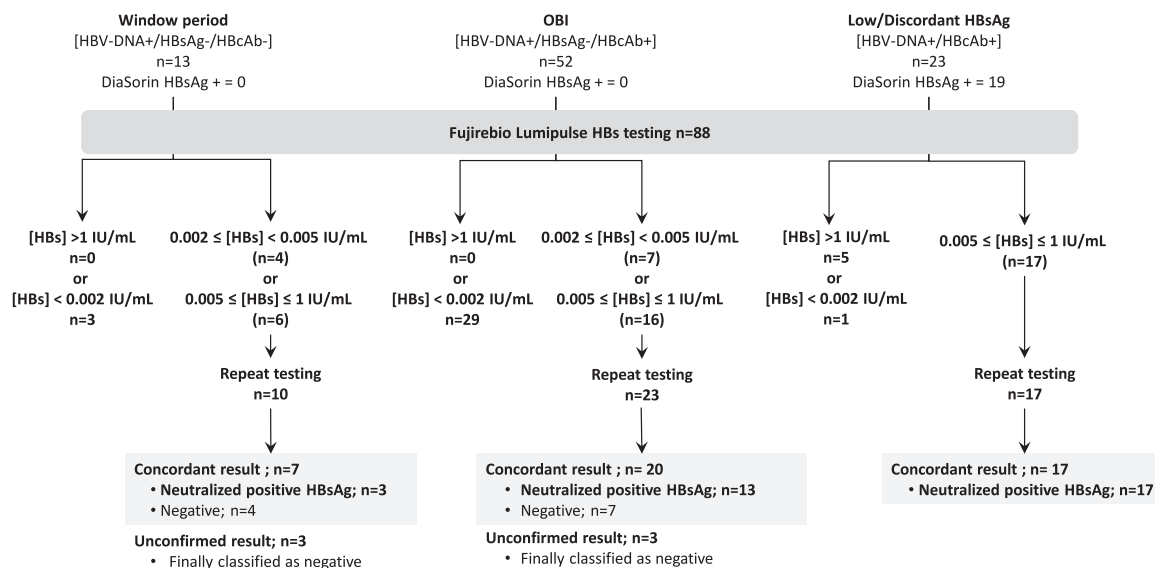


Fig. 1. Study flowchart and associated results.

from 0.05 to 5,100 IU/mL. In contrast, 39 (44 %) samples tested positive (HBsAg \geq 0.005 IU/mL) on initial testing with the Fujirebio assay. All negative or positive results with HBsAg concentrations between 0.002 and 1 IU/mL on initial testing (50 samples, 57 %) were retested in a second run. Six (12 %) samples initially found positive were finally classified as HBsAg-negative after two negative results on repeat testing (referred as "unconfirmed result" in Fig. 1). The first results were confirmed in repeat assays for 44 (88 %) samples (11 as HBsAg-negative and 33 as HBsAg-positive). All samples which were repeatedly HBsAg-positive (n = 33) were further tested using the confirmatory inhibition assay and were confirmed as true positives with a mean inhibition rate of 83 % (range 67–93 %).

The characteristics of the reactive samples detected solely by Fujirebio are summarized in Table 2. The three samples from panel 1 with confirmed positive HBsAg results were HBV-DNA-positive, with viral loads ranging from 251 to 456 IU/mL. (Details on 3 anti-HBs positive samples are provided in the supplementary file).

In panel 2, the 13 samples with confirmed positive Fujirebio HBsAg results had HBsAg concentrations ranging from 0.005 to 0.057 IU/mL. All samples were positive for HBV-DNA (range: detected below 10 IU/

Table 2
Characteristics of the samples detected solely with the Fujirebio assay.

Panel	HBsAg Fujirebio [IU/mL]	HBsAg DiaSorin [IU/mL]	HBV-DNA [IU/mL]	Anti-HBs Ab [IU/L]	Genotype
1-Window Period	0.016	< 0.03	456	Negative	E
	0.016	< 0.03	251	Negative	A
	0.018	< 0.03	284	Negative	A
2-OBI	0.005	< 0.03	33	Negative	A
	0.006	< 0.03	106	Negative	C
	0.010	< 0.03	163	Negative	E
	0.010	< 0.03	23	11	B
	0.011	< 0.03	Detected (BQL)	Negative	D
	0.012	< 0.03	11	Negative	B
	0.012	< 0.03	13	Negative	A
	0.014	< 0.03	16	Negative	Not tested
	0.017	< 0.03	267	Negative	A
	0.026	< 0.03	3670	225	C
	0.043	0.048	372	Negative	A
	0.054	0.049	Detected (BQL)	Negative	Not tested
	0.057	0.046	16	Negative	A

BQL: below quantification limit of the assay.

mL and 3,670 IU/mL) and 2 of them were also positive for anti-HBs antibodies. It can be noted that all HBsAg levels measured with the Fujirebio assay were below or around the LoQ of the DiaSorin assay. The highest values measured with the Fujirebio assay corresponded to values close to the LoQ of the DiaSorin assay. All main genotypes were represented (A–E). Anti-HBs status was available for 85 samples, 58 (65.9 %) being negative and 27 (30.7 %) positive. The performance of the two assays was compared according to anti-HBs status. For anti-HBs negative samples, a significantly higher HBsAg-positive rate was observed with the Fujirebio assay (n = 31, 53 %) compared to the DiaSorin assay (n = 14, 24 %) (p < 0.0001). In contrast, no significant difference was observed for the 27 anti-HBs positive samples with comparable positive rates of 18 % and 26 % (p = 0.16), for DiaSorin and Fujirebio, respectively.

In the low/discordant group, 6 samples were anti-HBc and anti-HBs negative including 2 samples that were HBV-DNA-negative, corresponding to an HBsAg positivity following an HBV vaccination. The 4 remaining were DNA positive with an available genotype for 3 of 4. These 6 samples were HBsAg-positive with the Fujirebio assay while only four samples were HBsAg-positive with the DiaSorin assay. Six other samples in this group were anti-HBs low-positive (11–95 IU/L). Five of these samples were HBsAg-positive in both assays studied while one was HBsAg-negative in both tested assays.

Of the 88 samples tested, 18 were within the quantification range of both tests. Comparisons of HBsAg quantification were performed on the 16 samples with values below 2 IU/mL to assess the accuracy of the two techniques in the lower dynamic range. All samples belonged to panel 3. As shown in Fig. 2, the Spearman correlation coefficient between the two techniques was 0.893 with a slope of 1.69. The Bland-Altman analysis indicated that HBsAg concentrations measured using Fujirebio were slightly higher than those measured using DiaSorin (mean bias of 0.17 IU/mL) with a quantification difference tending to decrease towards lower HBsAg values.

4. Discussion

In our study, we evaluated the performances of the Fujirebio assay in detecting low HBsAg levels in clinical samples and compared it to the DiaSorin quantitative HBsAg assay used in our routine practice. The enhanced sensitivity of the Fujirebio assay was confirmed in three different types of samples. Almost a quarter of documented HBsAg-negative samples, as determined using conventional assays from panels 1 and 2 (16/65 (25 %)) were finally identified as HBsAg-positive with

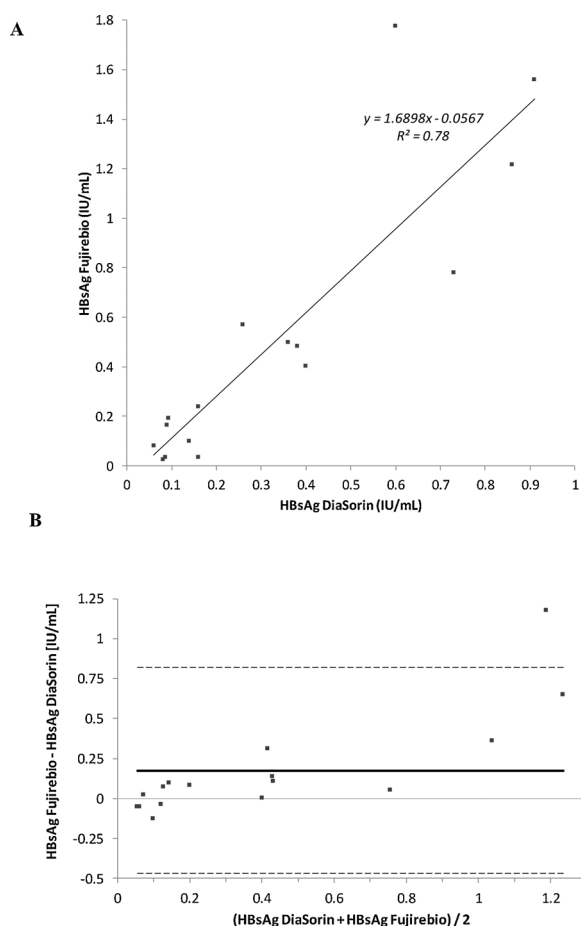


Fig. 2. A: Correlation between serum HBsAg concentrations (below 2 IU/mL) quantified using the two assays; B: Bland-Altman analysis of HBsAg concentrations measured using Fujirebio and DiaSorin assays. The solid line represents the mean difference between the assays. The dashed line illustrates the 95 % confidence interval of the mean difference.

the Fujirebio assay. All of these samples were HBV-DNA-positive with an HBsAg reactivity confirmed by neutralization, attesting specificity. It is essential to repeat the test for each case of HBsAg reactivity, as recommended, in particular in case of low HBsAg titers, to ascertain the specificity of the assay. Indeed, in this set, 6 initially HBsAg-reactive samples were finally not confirmed as positive.

Across panels 2 and 3, 56 samples corresponded to a serological profile of past infection (HBsAg-negative / anti-HBc-positive) as determined with the DiaSorin assay (Table 2). The HBsAg positivity rate increased from 34 % (19/56) to 63 % (35/56) when tested with our routine technique and the more sensitive Fujirebio assay respectively. Using a highly sensitive screening test, a patient formerly identified as functionally cured can be reclassified as a chronic carrier with low HBsAg concentrations. The clinical consequences of these findings may be limited, as low HBsAg concentrations are generally predictive of an inactive status and most of these patients do not require medical attention [5]. Considering solely identified OBI patients (panel 2, n = 52), 25 % (13/52) of the patients would have been reclassified as chronic carriers using the Fujirebio assay. Thus, patients classified as OBI with the current assays could be reclassified as chronic carriers using more sensitive HBsAg or molecular assays [8,17]. Although the clinical consequences of OBI remain debated, changing the patient's status from HBsAg-negative to positive will certainly modify both patient and physician perceptions of the infection. For the remaining OBI samples (n = 39) with undetectable HBsAg, the possibility of even lower HBsAg concentrations is challenging.

In some cases, a low level HBsAg is associated with low to moderate replication, compatible with transmission, at least by transfusion [18]. In terms of public health, the identification of these HBsAg carriers is therefore highly desirable. In high-prevalence countries where NAT is not implemented for blood screening, improving HBsAg detection sensitivity tenfold would significantly reduce the WP thereby limiting HBV transmission. Although we only tested 13 samples corresponding to the WP, 3 (23 %) were identified as positive, confirming the potential added value of these more sensitive assays for blood screening. Matsubara et al. tested a sensitive immunoassay on seroconversion panels [19]. The mean time to HBsAg first detection of HBsAg was 17.4 days, less than the delay obtained with existing assays including PRISM. In the ongoing effort to improve HBsAg assays, Abbott recently described a new ultrasensitive qualitative prototype assay with clinical performance approaching that of the NAT mini-pool and reducing the early WP as shown on seroconversion panels [15]. Recently, studying profiles of two longitudinal vaccine breakthrough from plasma donors, Kuhns et al. showed that the prototype significantly reduced the WP with HBsAg detection 21 and 43 days earlier than with currently used HBsAg assays [20]. Our data and these studies clearly indicate the benefit of more sensitive HBsAg detection in the context of blood safety for countries that may not be able to afford NAT.

Our study strengthens previous studies exploring quantification comparisons between techniques. In a study assessing the performance of 7 quantitative HBsAg assays on the WHO International Standard and a regional reference panel [21], two kits, BLEIA Eiken HBsAg (BLEIA; Eiken Chemical, Tokyo, Japan) and Fujirebio, detected HBsAg concentrations of 0.010 IU/mL. For samples with low HBsAg concentrations (< 2 IU/mL) we demonstrate that the Fujirebio and DiaSorin assays provide similar values on different HBV genotypes with a mean bias of 0.17 IU/mL. These findings are in line with a previous study comparing the Lumipulse (Fujirebio) with the Architect (Abbott) and the Elecsys (Roche) assays in various clinical settings [22].

Depending on the assay, different HBsAg-positive rates according to the patient's anti-HBs status were noted in our study. The coexistence of HBsAg and anti-HBs raises the question of the effect of anti-HBs on the analytical sensitivity of HBsAg assays [12]. In previous studies, re-examination of samples from HBV carriers in whom HBsAg seroclearance had been confirmed using conventional assays showed a positive HBsAg result with the Fujirebio assay, months or years away from the initial seroclearance, with differences according to the anti-HBs status [13,23,24]. Negative anti-HBs status, and also antiviral therapy and liver cirrhosis seem to contribute to the discrepancies between ultrasensitive and conventional assays in multivariate analyses [24]. As functional cure and the decision to stop antiviral treatment are based on the disappearance of HBsAg from the serum, more sensitive HBsAg assays will require extensive clinical validation to assess their impact on clinical management [7,25].

Improved HBsAg assay sensitivity could also modify the risk of reactivation assessment under immunosuppressive therapies. This risk seems greater if HBsAg is detected [26]. Among 120 HBV-resolved patients receiving chemotherapy, 12 had quantifiable HBV-DNA. HBsAg was detected using the Fujirebio assay in all reactivating patients [27]. Thus, detection of HBsAg with more sensitive assays among patients previously identified as HBsAg-negative would certainly lead to different clinical management.

This study clearly indicates that more sensitive HBsAg assays will significantly improve screening and blood transfusion safety. In clinical management, the gain in HBsAg sensitivity will probably translate into an adjustment of treatment follow-up and patient classification according to their serological profiles. With these improved assays, one must be prepared to reclassify patients initially documented as OBI or functionally cured as chronic carriers with low HBsAg levels.

CRediT authorship contribution statement

Charlotte Pronier: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Daniel Candotti:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing. **Laure Boizeau:** Investigation, Resources, Data curation. **Jérémy Bomo:** Validation, Investigation, Resources, Data curation, Visualization. **Syria Laperche:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision. **Vincent Thibault:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision.

Declaration of Competing Interest

None.

Acknowledgment

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jcv.2020.104507>.

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