Clinical Trials Study

Hepatitis B surface antigen and hepatitis B core-related antigen kinetics after adding pegylated-interferon to nucleos(t)ids analogues in hepatitis B e antigen-negative patients

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Abstract

Background
Hepatitis B e antigen-negative chronic hepatitis B patients under nucleos(t)ids analogues (NAs) rarely achieve hepatitis B surface antigen (HBsAg) loss.

Aim
To evaluate if the addition of pegylated interferon (Peg-IFN) could decrease HBsAg and hepatitis B core-related antigen (HBcrAg) levels and increase HBsAg loss rate in patients under NAs therapy.

Methods
Prospective, non-randomized, open-label trial evaluating the combination of Peg-IFN 180 µg/week plus NAs during forty-eight weeks vs NAs in monotherapy. Hepatitis B e antigen-negative non-cirrhotic chronic hepatitis B patients of a tertiary hospital, under NAs therapy for at least 2 years and with undetectable viral load, were eligible. Patients with hepatitis C virus, hepatitis D virus or human immunodeficiency virus co-infection and liver transplanted patients were excluded. HBsAg and HBcrAg levels (log10 U/mL) were measured at baseline and during ninety-six weeks. HBsAg loss rate was evaluated in both groups.
Adverse events were recorded in both groups. The kinetic of HBsAg for each treatment group was evaluated from baseline to weeks 24 and 48 by the slope of the HBsAg decline (log10 IU/mL/week) using a linear regression model.

**RESULTS**
Sixty-five patients were enrolled, 61% receiving tenofovir and 33% entecavir. Thirty-six (55%) were included in Peg-IFN-NA group and 29 (44%) in NA group. After matching by age and treatment duration, baseline HBsAg levels were comparable between groups (3.1 vs 3.2) \( (P = 0.25) \). HBsAg levels at weeks 24, 48 and 96 declined in Peg-IFN-NA group (-0.26, -0.40 and -0.44) and remained stable in NA group (-0.10, -0.10 and -0.10) \( (P < 0.05) \). The slope of HBsAg decline in Peg-IFN-NA group (-0.02) was higher than in NA group (-0.00) \( (P = 0.015) \). HBcrAg levels did not change. Eight (22%) patients discontinued Peg-IFN due to adverse events. The HBsAg loss was achieved in 3 (8.3%) patients of the Peg-IFN-NA group and 0 (0%) of the NA group.

**CONCLUSION**
The addition of Peg-IFN to NAs caused a greater and faster decrease of HBsAg levels compared to NA therapy. Side effects of Peg-IFN can limit its use in clinical practice.

**Key Words:** Chronic hepatitis B; Hepatitis B e antigen-negative; Hepatitis B surface antigen; Hepatitis B core-related antigen; Pegylated-interferon; Nucleos(t)ids analogues

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**INTRODUCTION**

Chronic hepatitis B (CHB) affects around 240 million people worldwide[^1]. Hepatitis B virus (HBV) cannot be completely eradicated with the available therapies due to the presence of covalently closed circular DNA (cccDNA) in the nuclei of infected hepatocytes[^1]. Hepatitis B surface antigen (HBsAg) loss is the optimal treatment endpoint, representing a functional cure of CHB and improving long-term outcome[^3].

Although liver biopsy for the quantification of intrahepatic cccDNA and intrahepatic HBV DNA remains the most accurate measurement for viral reservoir, it is limited by its invasive nature and the potential for sampling error. Therefore, noninvasive serological tests are necessary as surrogate markers of intrahepatic viral replicative activity. Serum HBsAg is the glycosylated envelope protein of the mature HBV, which is produced by transcription and translation of the surface genes[^5]. On the other hand, the hepatitis B core-related antigen (HBcrAg) combines the antigenic reactivity resulting from denatured hepatitis B e antigen (HBeAg), HBV core antigen and a core-related protein (p22cr), all products of the precore/core gene share an

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[^2]: https://www.wjgnet.com/1948-5182/full/v12/i11/1076.htm
[^3]: doi: https://dx.doi.org/10.4254/wjh.v12.i11.1076
HBsAg kinetics after adding pegylated-interferon

MATERIALS AND METHODS

Patients and study design
This is a single center, prospective, non-randomized, open-label trial including HBeAg-negative non-cirrhotic CHB patients, receiving NAs for at least 2 years. Recruitment period was from August 2014 to February 2016 in a tertiary center (Hospital del Mar, Barcelona, Spain). Patients were eligible if they received a stable NAs dose with virological response (undetectable HBV-DNA viral load during the last twelve months). Exclusion criteria were as follows: Patients with a previous Peg-IFN treatment, NA treatment for HBV reactivation prophylaxis, patients with human immunodeficiency virus, hepatitis D virus or hepatitis C virus co-infection, and liver transplanted patients. All patients provided written informed consent.

Patients with any malignancy in the last 5 years, those with psychiatric, thyroid or autoimmune disorders, and non-liver transplanted patients were only eligible for NAs monotherapy. Peg-IFN alpha-2a was offered to be added in all eligible patients. Those who accepted it, received 180 µg/week during forty-eight weeks (Peg-IFN-NA group) and all the other participants remained in NAs monotherapy (NA group). At week 48 all the patients continued with NAs in monotherapy and were followed up until week 96 or loss of follow-up. Protocol visits were at weeks 0, 12, 24, 48, 72 and 96. Figure 1 shows the flowchart of patients and study design.

The study protocol was approved by the Ethical Committee of our Institution “Comité Ètic d’Investigació Clínica-Parc de Salut Mar”, study reference 2014/5787/I, in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Clinical variables and definitions
Demographic data, liver stiffness measurement (LSM) and polymorphism rs12979860 of IL28B were assessed at baseline. HBV-genotype was collected from electronic data as it had been performed prior to the initiation of NAs therapy. The levels of HBV-DNA, HBsAg and HBcAg were analyzed at weeks 0, 24, 48, 96. Adverse events were recorded at each protocol visit, following the Common Terminology Criteria for Adverse Events. All the data were collected and tabulated in a database with an access code to ensure patient confidentiality.

LSM was performed at baseline by a single experienced operator (> 5000 examinations), using the FibroScan® 502 Touch (FibroScan® EchosensTM, Paris, France) following the manufacturer’s recommendations as previously described[8]. Liver fibrosis was categorized according to previously published cut-offs for LSM considering significant fibrosis for LSM > 7.2 kPa. Patients with LSM > 12 kPa were considered as having cirrhosis and were excluded[9].
**Virological markers**
HBV DNA was measured by polymerase chain reaction with a limit of quantification of 10 IU/mL (ABBOTT RealTime HBV m2000®, Abbott Molecular Inc., IL, United States). Serum HBsAg was quantified by Electro-chemiluminescence immunoassay Elecsys® HBsAgII QuantII (Roche Diagnostic, Rotkreuz, Switzerland) according to the manufacturer’s instructions. The assay ranged from 0.05 to 117000 IU/mL. In highly concentrated samples above the upper limit, the value of manual dilution was multiplied by the dilution factor. Serum HBcAg was measured using a quantitative fully automated chemiluminiscent enzyme immunoassay (LUMIPULSE®, Fujirebio Europe, Belgium).

The monoclonal antibodies used in this two-step immunoassay measure simultaneously denatured HBeAg, HBV core antigen and the precore protein p22cr (aa-28 to aa-150). Samples were processed according to the manufacturer’s instructions. The lower limit of detection was 2.0 log U/mL, and a linear range of 3.0 log U/mL to 7.0 log U/mL (1 kU/mL was equal to 3 log U/mL).

**Statistical analysis**
Quantitative variables were expressed as medians and ranges. Categorical variables were expressed as proportions. Continuous variables were compared by the Mann-Whitney U test or Kruskall-Wallis when appropriate and categorical by the Pearson chi-square test, Fisher test or the McNemar test. Patients were categorized according to antiviral treatment (Peg-IFN-NA group vs NA group). Differences between NA and Peg-IFN-NA groups regarding age, sex, IL28B polymorphism, ethnicity, liver function, liver stiffness, treatment duration, viral genotype, HBsAg and HBcAg levels and HBsAg loss rate were analyzed by univariate analysis. A two-sided P value < 0.05 was considered to indicate statistical significance. The kinetic of HBsAg for each treatment group was evaluated from baseline to weeks 24 and 48 by the slope of the HBsAg decline (log10 IU/mL per week) using a linear regression model (LRM). Statistical analyses were performed with the SPSS® 25.0 (SPSS Inc., Chicago, IL, United States) and LRM with the Prism 7.0 (© 1994-2016 GraphPad Software, Inc.).

**RESULTS**

**Study population and baseline characteristics**
From August 2014 to February 2016, 119 HBeAg-negative CHB patients were evaluated. Twenty-nine (24%) patients declined their participation, 10 (8.4%) had
Previously received Peg-IFN, 10 (8.4%) had liver cirrhosis and in 5 (4.2%) patients NAs therapy duration was shorter than 2 years. Among the 65 included patients, 5 were only eligible for the NA therapy due to Peg-IFN contraindications and 60 were eligible for both therapies: 36 accepted to receive Peg-IFN and 24 refused the addition of Peg-IFN. Therefore, 36 (55.4%) patients were included in the Peg-IFN-NA group and 29 (44.6%) in the NA group. Two patients in NA group were receiving low doses of corticosteroids (prednisone 2.5 to 5 mg/d) for rheumatoid arthritis and no kidney transplanted patients were included because none of them fulfilled the inclusion criteria.

Figure 1 shows the flowchart and Table 1 the main characteristics of the included patients. Patients in Peg-IFN-NA group compared to NA group were younger (age 45 vs 53, P = 0.01) and had a shorter previous NA treatment duration (259 vs 393 wk, P = 0.01), but were comparable in gender, IL28B polymorphism, ethnicity, liver function, liver stiffness, type of NA, HBV genotype and baseline HBcAg and HBsAg levels. Due to the baseline differences, patients of both treatment groups were individually matched for age and treatment duration. Therefore, pre-treatment predictors and the kinetic of serological markers (HBsAg and HBcAg) were performed in 48 patients. Table 2 shows the characteristics of matched patients.

**HBcAg kinetics according to baseline variables and treatment group**

The median (range) HBcAg values (log 10 U/mL) was 2.7 (< 2-4.9) in NA group and 2.3 (< 2-3.7) in Peg-IFN-NA group (P = 0.18) at baseline. The rate of patients with HBcAg values below the limit of detection (HBcAg < 2 log10 U/mL) was 25% and 38%, respectively (P = 0.39). The HBcAg kinetics was described as the delta (Δ) of its levels at weeks 24, 48 and 96. The HBcAg levels remained stable at weeks 24, 48 and 96 (Table 2). We did not detect differences on HBcAg levels between both treatment strategies according to the treatment group, the IL28B polymorphism or the HBV genotype. We did not find any correlation between HBcAg and HBsAg levels nor HBsAg loss rate (data not shown).

**HBsAg kinetics according to baseline variables and treatment group**

The baseline levels of HBsAg (log 10 IU/mL) were similar in NA and Peg-IFN-NA groups (3.1 vs 3.2) (P = 0.25). The HBsAg kinetics was described as the delta (Δ) of their levels at weeks 24, 48 and 96. The decline of the HBsAg level was greater in Peg-IFN-NA group (-0.26, -0.40, -0.44) compared to NA group (-0.11, -0.10, -0.12) (P < 0.05 in all determinations) (Figure 2).

The HBsAg kinetics was different between treatment arms according to IL28B polymorphism and HBV genotype. In patients with IL28B CC polymorphism (n = 22) the decline of HBsAg at weeks 24, 48 and 96 was greater in Peg-IFN-NA group (-0.27, -0.92 and -0.64) than in NA group (-0.11, -0.11 and -0.10) (P < 0.05 in all cases) (Figure 3A). In contrast, in patients with IL28B CT/TT (n = 26) we did not find differences on HBsAg kinetics at weeks 24, 48 and 96 between Peg-IFN-NA group (-0.09, -0.11 and -0.19) and NA group (-0.10, -0.07 and 0.13) (not significant in all determinations) (Figure 3B). Moreover, the decline of HBsAg were different between NA and Peg-IFN-NA group at weeks 48 and 96 in patients infected by HBV genotype A (-0.07 vs -1.05 and -0.08 vs -0.53) and genotype D (-0.08 vs -0.42 and -0.51 vs -0.80) (P < 0.05 in all cases) (data not shown).

**LRM to recognize different HBsAg kinetics**

In order to demonstrate the existence of different HBsAg kinetics for each treatment strategy, we evaluated the slope of the HBsAg decline (log10 IU/mL per week) from baseline to weeks 24 and 48 using a LRM (Figure 4). In patients receiving NA monotherapy, HBsAg levels did not decrease during the forty-eight weeks. The slope of HBsAg kinetics in NA group (-0.00) was similar to zero (P = 0.6). On the contrary, in patients receiving Peg-IFN-NA, HBsAg levels significantly decreased during the forty-eight weeks and the slope of HBsAg kinetic (-0.02) was different to zero (P < 0.001) and greater than that found in NA group (P = 0.015).

**Rate of low HBsAg levels and HBsAg loss during follow-up**

The proportion of patients reaching low levels of HBsAg (HBsAg < 100 IU/mL) at baseline and at weeks 24, 48 and 96 are depicted in Figure 5. In the NA group the rate of patients with low HBsAg levels was 21% at baseline, but did not change at weeks 24, 48 and 96 (not significant) (Figure 5A). On the contrary, rate of patients with low HBsAg levels in Peg-IFN-NA group was 4.2% at baseline and increased at weeks 24 (16.7%), 48 (29.6%) and 96 (16.7%) (P = 0.001) (Figure 5B). The proportion of patients...
Table 1 Main characteristics of the included patients

|                           | NA group (n = 29) | Peg-IFN-NA group (n = 36) | P value |
|---------------------------|------------------|--------------------------|---------|
| Age (yr)                  | 53 (36-70)       | 45 (26-72)               | 0.01    |
| Males, n (%)              | 21 (72)          | 29 (81)                  | 0.44    |
| IL28B polymorphism, n (%) |                  |                          | 0.16    |
| CC                        | 11 (37.9)        | 20 (55.6)                |         |
| CT/TT                     | 14 (62.1)        | 16 (44.4)                |         |
| Origin (ethnicity), n (%) |                  |                          | 0.70    |
| Europe                    | 20 (69)          | 20 (56)                  |         |
| Asia                      | 12 (33)          | 12 (33)                  |         |
| Africa                    | 3 (10)           | 3 (8)                    |         |
| AST (IU/mL)               | 20 (15-59)       | 22 (12-62)               | 0.37    |
| ALT (IU/mL)               | 19 (12-101)      | 25 (12-91)               | 0.20    |
| GGT (IU/mL)               | 19 (9-197)       | 22 (10-125)              | 0.33    |
| LSM, n (%)                |                  |                          | 0.91    |
| < 7.2 kPa                 | 28 (97)          | 34 (97)                  |         |
| 7.2-12 kPa                | 1 (3)            | 1 (3)                    |         |
| NA treatment, n (%)       |                  |                          |         |
| Tenofovir                 | 20 (69)          | 22 (61)                  | 0.46    |
| Entecavir                 | 7 (24)           | 11 (31)                  |         |
| Others                    | 2 (7)            | 3 (8)                    |         |
| NA treatment duration (wk)| 393 (113-763)    | 259 (118-496)            | 0.01    |
| HBV genotype, n (%)       |                  |                          | 0.99    |
| Non-D                     | 7 (24.1)         | 16 (44.4)                |         |
| D                         | 12 (41.4)        | 13 (36.1)                |         |
| Not available             | 10 (34.5)        | 7 (19.4)                 |         |
| Baseline HBcrAg (log 10 U/mL) | 2.65 (< 2.4.9) | 2.30 (< 2.3.7)          | 0.18    |
| Baseline HBsAg (log 10 IU/mL) | 2.96 (1.3-4.2) | 3.22 (1.6-4.6)          | 0.07    |

Quantitative variables are expressed as median (range); qualitative variables are expressed as n (%). NA: Nucleos(t)id analogue; Peg-IFN: Pegylated interferon; IL28B: Interleukin 28B; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; LSM: Liver stiffness measurement; HBV: Hepatitis B virus; HBcrAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen.

Achieving HBsAg loss in the Peg-IFN-NA group (n = 3, 12.5%) was higher compared to NA group (n = 0, 0%), but the difference did not reach the statistical significance (P = 0.07). Patients with HBsAg loss were male, with low fibrosis stage (F0-F1), and infected by HBV-genotype A (n = 1) or B (n = 2). Two patients had an IL28B CC polymorphism and the other a CT polymorphism. All of them had been on NAs therapy for more than 5 years before the addition of Peg-IFN. The NAs treatment was entecavir (n = 1), tenofovir (n = 1) and telbivudine (n = 1). Baseline levels of HBsAg (log10 IU/mL) were 4.0, 2.1 and 1.6, and baseline levels of HBcrAg (log10 U/mL) were 2.7, < 2 and 3.4, respectively. All of them received Peg-IFN during forty-eight weeks. Two patients lost HBsAg during therapy (week 24 and 36) and one at week 24 after Peg-IFN discontinuation (week 72).

Safety
No serious adverse events were observed during treatment and follow-up. However, 8 (22%) patients did not complete Peg-IFN treatment. The reasons for Peg-IFN discontinuation were flu-like symptoms and asthenia (n = 3), DNA flare (n = 3),
| Characteristics                                                                 | NA group (n = 24) | Peg-IFN-NA group (n = 24) | P value |
|---------------------------------------------------------------------------------|------------------|--------------------------|---------|
| **Age (yr)**                                                                    | 54 (36-60)       | 45 (26-63)               | 0.07    |
| **Male sex, n (%)**                                                             | 18 (75)          | 22 (91)                  | 0.12    |
| **IL28B polymorphism, n (%)**                                                   |                  |                          | 0.25    |
| CC                                                                              | 9 (38)           | 13 (54)                  |         |
| CT/CT                                                                           | 15 (62)          | 11 (46)                  |         |
| **Origin (ethnicity), n (%)**                                                    |                  |                          | 0.20    |
| European                                                                        | 17 (70)          | 12 (50)                  |         |
| Asia                                                                            | 3 (12)           | 9 (38)                   |         |
| Africa                                                                          | 2 (8)            | 3 (12)                   |         |
| **AST (IU/mL)**                                                                 | 20 (15-59)       | 22 (15-38)               | 0.69    |
| **ALT (IU/mL)**                                                                 | 20 (12-101)      | 23 (15-50)               | 0.41    |
| **GGT (IU/mL)**                                                                 | 23 (9-197)       | 22 (11-125)              | 0.44    |
| **LSM, n (%)**                                                                  |                  |                          | 0.32    |
| < 7.2 kPa                                                                       | 23 (96)          | 24 (100)                 |         |
| 7.2-12 kPa                                                                      | 1 (4)            | 0 (0)                    |         |
| **NA treatment, n (%)**                                                          |                  |                          | 0.32    |
| Tenofovir                                                                       | 16 (67)          | 12 (50)                  |         |
| Entecavir                                                                       | 6 (25)           | 9 (38)                   |         |
| Others                                                                          | 2 (8)            | 3 (12)                   |         |
| **NA treatment duration (wk)**                                                   | 378 (113-763)    | 272 (139-495)            | 0.06    |
| **HBV genotype, n (%)**                                                          |                  |                          | 0.43    |
| A                                                                               | 5 (21)           | 4 (17)                   |         |
| B                                                                               | 1 (4)            | 3 (12)                   |         |
| C                                                                               | 0 (0)            | 2 (8)                    |         |
| D                                                                               | 10 (42)          | 8 (33)                   |         |
| E                                                                               | 1 (4)            | 2 (8)                    |         |
| F                                                                               | 0 (0)            | 1 (4)                    |         |
| Not available                                                                   | 7 (29)           | 4 (18)                   |         |
| **Baseline HBcrAg (log 10 U/mL)**                                               | 2.7 (< 2-4.9)    | 2.3 (< 2-3.7)            | 0.18    |
| **Baseline HBsAg (IU/mL), n (%)**                                               |                  |                          | 0.39    |
| < 2                                                                             | 6 (25)           | 9 (38)                   |         |
| 2-2.5                                                                           | 4 (17)           | 6 (25)                   |         |
| 2.5-3                                                                           | 6 (25)           | 3 (12)                   |         |
| 3-3.5                                                                           | 2 (8)            | 3 (12)                   |         |
| 3.5-4                                                                           | 3 (13)           | 3 (12)                   |         |
| > 4                                                                             | 3 (13)           | 0 (0)                    |         |
| **Baseline HBsAg (log10 U/mL)**                                                 | 3.1 (1.3-4.2)    | 3.2 (1.6-4.4)            | 0.25    |
| **Baseline HBsAg (IU/mL), n (%)**                                               |                  |                          | 0.22    |
| > 1000                                                                          | 12 (50)          | 14 (48)                  |         |
| 100-1000                                                                        | 7 (29)           | 9 (38)                   |         |
| < 1000                                                                          | 5 (21)           | 1 (4)                    |         |
HbcAg decline (log10 U/mL)

|                | Δ Week 24       | Δ Week 48       | Δ Week 96       |
|----------------|-----------------|-----------------|-----------------|
|                | 0.00 (-1.10-1.21) | 0.00 (-0.71-0.30) | 0.96            |
|                | 0.00 (-1.00-0.30) | 0.00 (-1.31-1.10) | 0.25            |
|                | 0.00 (-1.00-0.10) | 0.00 (-0.71-0.71) | 0.12            |

HBsAg decline (log10 IU/mL)

|                | Δ Week 24       | Δ Week 48       | Δ Week 96       |
|----------------|-----------------|-----------------|-----------------|
|                | -0.11 (-0.04-0.00) | -0.26 (-3.8-0.1) | 0.01            |
|                | -0.10 (-1.17-0.04) | -0.40 (-4.02) | 0.00            |
|                | -0.12 (-1.39-0.96) | -0.44 (-4.01) | 0.00            |

HBsAg Loss; n (%)

|                | 0 (0) | 3 (12.5) | 0.07 |

Quantitative variables are expressed as median (range); qualitative variables are expressed as n (%). NA: Nucleos(t)id analogue; Peg-IFN: Pegylated interferon; IL28B: Interleukin28B; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; LSM: Liver stiffness measurement; HBV: Hepatitis B virus; HbcAg: Hepatitis B core related antigen; HBsAg: Hepatitis B surface antigen.

**DISCUSSION**

In this controlled trial of HBeAg-negative CHB non-cirrhotic patients under NAs treatment and with undetectable DNA, the addition of 48 wk of Peg-IFN alfa-2a reduced HBsAg levels further and faster than continuing with NAs monotherapy. However, the proportion of patients with HBsAg loss during the first ninety-six weeks did not reach the statistical significance with this add-on strategy.

HBsAg kinetics has been shown as one of the best predictors of treatment response\[8,16,17\]. However, patients of our Peg-IFN-NA group were younger and had a shorter previous NA treatment duration compared to NA group. According to previously published studies showing a decrease of HBsAg levels with NA therapy\[18\] and a higher probability to HBsAg clearance in aged populations\[19\] we decided to match the included patients for age and treatment duration.

The present study prospectively confirms our previously published results\[7\] regarding the slow decline of HBsAg levels in HBeAg-negative CHB patients receiving NAs therapy. The current study has demonstrated a very low decline (-0.12 log10 IU/mL at week 96) and very slow change (-0.00 log10 IU/mL per week) of HBsAg levels in patients receiving NAs. As a consequence, the rate of patients with low HBsAg levels (< 100 IU/mL) did not change at weeks 24, 48 and 96, and no patient achieved HBsAg loss. On the contrary, the addition of Peg-IFN clearly increased the
decline (-0.44 log10 IU/mL at week 96) and accelerate the decrease (-0.02 log10 IU/mL per week) of HBsAg levels compared to NA group. Therefore, in the Peg-IFN-NA group the rate of patients with low HBsAg levels was higher at weeks 24 (16.7%) and 48 (29.6%) and the rate of HBsAg loss increased (n = 3, 12.5%) compared to NA group (n = 0, 0%).

We also analyzed the HBeAg levels during the study in both treatment strategies. However, levels of HBeAg remained stable during the 96 wk without differences between both treatment strategies and without correlation with HBsAg levels or HBsAg loss rate. This could be explained by the fact that baseline levels of HBeAg in our cohort of HBeAg negative patients, receiving NAs during a long time period before inclusion, were already low. As described before, the rate of patients with a baseline HBeAg value below the limit of detection (< 2 log10 U/mL) was high in both treatment groups (25% and 38%). Recent studies have shown that HBeAg can reflect cccDNA transcriptional activity in the different phases of HBV infection\textsuperscript{20,21}. However as HBeAg is included in HBcAg, this could explain the low baseline HBcAg levels in our cohort of HBeAg-negative patients. Moreover, recent studies, have described that...
HBcrAg levels can decline over the time in patients undergoing NAs therapy, especially in HBeAg-negative patients\(^{22,23}\). Thus, according to our results, we have not found that HBcrAg determination could be a useful serum marker in clinical practice for monitoring treatment response in HBeAg-negative patients receiving NAs or Peg-IFN-NAs.

It has been suggested that low levels of HBsAg are related to higher rates of HBsAg loss after NA discontinuation, being advisable to achieve low levels of HBsAg before stopping NA therapy\(^{24,25}\). Our study showed that the rate of patients with HBsAg < 100 IU/mL increased in the Peg-IFN-NA group from 4.2% at baseline to 29.6% at 48 wk (\(P = 0.001\)). The NAs have shown to restore partly adaptive immunity, whereas Peg-IFN boosts innate immunity and depletes the ccc-DNA, which leads to a major HBsAg loss\(^{26-29}\). The analysis performed in matched patients by age and treatment duration showed that the proportion of HBsAg loss during the first 96 wk was higher in the Peg-IFN-NA group compared to the NA group. However, this difference did not reach the statistical significance probably due to the limited number of included patients and the short follow-up time of our study. Nevertheless, our results are in accordance with smaller studies previously published\(^{30,31}\) and in line with the results published by Bourlière et al\(^{32}\) during the execution of the current study.

Previous studies have linked the presence of IL28B CC polymorphisms with the HBsAg loss in HBeAg-negative CHB patients receiving Peg-IFN. It has been shown that CC polymorphism could confer a better response profile to Peg-IFN therapy than CT/TT polymorphisms, especially in patients infected by HBV genotype D\(^{11,33}\). We analyzed the HBsAg kinetics according to IL28B polymorphism, and we found that patients with CC polymorphism showed a higher HBsAg decline in Peg-IFN-NA group compared to NA group. On the contrary, HBsAg kinetics was similar in both treatment strategies in CT/TT patients. Therefore, the add-on strategy should not be recommended in patients with IL28B CT or TT polymorphism.

Our study has several limitations. First, the treatment assignment was not randomized. However, patients on both treatment strategies were individually matched for age and treatment duration to make the cohort comparable. Second, the acceptance of the add-on strategy was low and only 40% of eligible patients with a previous (well-tolerated) NA therapy accepted the addition of Peg-IFN due to its potential toxicity. Third, the frequent adverse events of Peg-IFN (22% of discontinuations) caused a low number of patients completing 48 wk of therapy making this therapeutic strategy difficult to be introduced in clinical practice. However, this applicability and tolerability are in line with previous published data\(^{33}\).

Fourth, the treatment duration of Peg-IFN was limited to 48 wk and the follow-up period to 96 wk. Therefore, patients with a rapid HBsAg decline could have taken advantage of a longer therapy or longer follow-up. Finally, the low rate of HBsAg loss did not allow to identify predictors associated with HBsAg loss. However, the LRM demonstrated different HBsAg kinetics after adding Peg-IFN.

Figure 5 Rate of patients with low hepatitis B surface antigen levels (Hepatitis B surface antigen < 100 IU/mL and 100-1000 IU/mL) according to treatment group. A: NA group; B: Peg-IFN-NA group. NS: Not significant; NA: Nucleos(t)ids analogue; Peg-IFN: Pegylated interferon.
CONCLUSION

In conclusion, our prospective, non-randomized, open-label clinical trial has demonstrated that the addition of Peg-IFN to NAs decreased HBsAg levels further and faster compared to NA monotherapy. The HBcrAg levels remained stable. Despite the low applicability and poor tolerance of Peg-IFN making difficult its use in clinical practice, it could be considered in selected patients with favorable HBV genotype and IL28B polymorphism.

ARTICLE HIGHLIGHTS

Research background
Functional cure of chronic hepatitis B (CHB), defined as the loss of hepatitis B surface antigen (HBsAg), is very unusual with current antiviral treatments in hepatitis B e antigen (HBeAg)-negative patients. HBsAg levels decline very slow in patients receiving nucleos(t)ides analogues (NAs). Therefore, they need long-term antiviral treatment.

Research motivation
The hypothesis that we wanted to answer with our study was that the addition of pegylated-interferon (Peg-IFN) could accelerate the decline of HBsAg levels in patients that were receiving NAs and that this therapeutic strategy could increase the HBsAg loss rate.

Research objectives
In our study we wanted to evaluate in patients under NAs therapy if the addition of Peg-IFN could decrease HBsAg and hepatitis B core-related antigen (HBcrAg) levels, and increase HBsAg loss rate. If HBeAg-negative patients could achieve low levels of HBsAg it could be a good strategy to shorten the antiviral treatment.

Research methods
We have performed a prospective, non-randomized, open-label trial evaluating the combination of Peg-IFN 180 µg/wk plus NAs during forty-eight weeks vs NAs in monotherapy, in HBeAg-negative non-cirrhotic CHB patients after a minimum of two years of NA therapy and with virological response.

Research results
We have shown that the addition of Peg-IFN 180 µg/wk during forty-eight weeks to NAs caused a greater and faster decrease of HBsAg levels compared to NA therapy alone, especially in those patients with interleukin 28B polymorphism CC. However, the HBcrAg levels remained stable after adding Peg-IFN to NAs. We have also shown that, the low acceptance by the patients of this therapeutic strategy and the side effects of Peg-IFN can limit its use in clinical practice.

Research conclusions
This study shows that the addition of Peg-IFN to NA therapy accelerates the decline of HBsAg, especially in patients with interleukin 28B polymorphism CC. Therefore, even Peg-IFN has several side effects, this treatment strategy could be offered to some selected patients in order to achieve the functional cure of CHB. On the other hand, our study shows that HBcrAg levels do not seem useful to monitor this kind of treatment, neither as a predictor of HBsAg loss.

Research perspectives
It is well known that patients with HBeAg-negative CHB usually need a long-term therapy with NAs, even lifelong, to achieve HBsAg loss. However, it has been suggested that low levels of HBsAg are related to higher rates of HBsAg loss after NA discontinuation, being advisable to achieve low levels of HBsAg before stopping NA therapy.
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