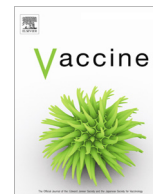




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Vaccination adjuvated against hepatitis B in Spanish National Healthcare System (SNS) workers typed as non-responders to conventional vaccines



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ABSTRACT

Summary: Trial Design: An interventional, phase 4, single group assignment, without masking (open label), preventive clinical trial was carried out in health workers with biological risk in their tasks, who have been filed as non-responders to conventional vaccination against Hepatitis B.

Methods: 67 health workers with biological risk in their tasks, who have been filed as non-responders to conventional vaccination against Hepatitis B, were enrolled in the Clinical Trial. All participants were from 18 years up to 64 years old. Inclusion Criteria: NHS workers -including university students doing their internships in health centres dependent on the National Health System (inclusion of students is regulated and limited by specific instructions on labour prevention in each autonomous community)- classified as non-responders. The criteria defining them as non-responders to the conventional hepatitis B vaccine is anti HBsAb titers < 10 mUI/ml following the application of six doses of conventional vaccine at 20 µg doses (two complete guidelines). The objective of this study was to provide Health workers-staff with an additional protection tool against hepatitis B infection, and to evaluate the efficacy of the adjuvanted vaccine in healthy non-responders to conventional hepatitis B vaccine. The primary outcome was the measurement of antibody antiHBs before the first Fendrix[®] dose and a month after the administration of each dose. Other outcome was collection of adverse effects during administration and all those that could be related to the vaccine and that occur within 30 days after each dose. In this study, only one group was assigned. There was no randomization or masking.

Results: The participants were recruited between April 13, 2018 and October 31, 2019. 67 participants were enrolled in the Clinical Trial and included the analyses. The primary immunisation consists of 4 separate 0.5 ml doses of Fendrix[®], administered at the following schedule: 1 month, 2 months and 6 months from the date of the first dose. Once the positivity was reached in any of the doses, the participant finished the study and was not given the following doses. 68.66% (46 out of 67) had a positive response to first

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dose of Fendrix[®]. 57.14% (12 out of 21) had a positive response to second dose of Fendrix[®]. 22.22% (2 out of 9) had a positive response to third dose of Fendrix and 42.96% (3 out of 7) had a positive response to last dose of Fendrix[®]. Overall, 94.02% (64 out of 67) of participants had a positive response to Fendrix[®]. No serious adverse event occurred.

Conclusions: The use of Fendrix[®], is a viable vaccine alternative for NHS workers classified as “non-responders”. Revaccination of healthy non-responders with Fendrix[®], resulted in very high proportions of responders without adverse events.

Trial registration: The trial was registered in the Spanish National Trial Register (REEC), ClinicalTrials.gov and inclusion has been stopped (identifier NCT03410953; EudraCT-number 2016-004991-23).

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1. Introduction

1.1. Scientific background and rationale

Independently of the current pandemic, working in health care entails exposure to numerous inherently associated risks even under normal circumstances. These include several biological risks which may vary widely according to the cases in question. One example is hepatitis B, which continues to cause many deaths worldwide.

Hepatitis B is the most serious type of viral hepatitis. It was first identified in 1963 by Blumberg, a US scientist. While analysing blood samples from populations around the world, he discovered the hepatitis B surface antigen in a sample from an Australian aborigine, hence he initially called it the Australian antigen. This laid the foundation for the study of a recently identified disease that had been known since ancient times as epidemic or catarrhal icterus [1–6]. Almost 20 years then passed until in 1981, the same Blumberg succeeded in obtaining a vaccine that was 95% effective against hepatitis B [7], followed by almost another decade of intensive vaccination campaigns until in 1991, the incidence of hepatitis B had fallen by approximately 82% in the United States [7–10].

Universal vaccination against this microorganism in children has improved in most developed countries. The vaccine has proved effective in reducing not only cases but also the long-term consequences of hepatitis B, such as chronic hepatitis, cirrhosis and hepatocellular carcinoma [2,6,9–12].

It is estimated that there are 250 million hepatitis B carriers in the world and that the virus kills about 900,000 people every year. According to the latest data from the European Union, the 25–44 age group presents the highest rate of new infections, followed by those aged 15–24. Prevalence in Europe ranges between 0.1% in Ireland and 4.4% in Romania. Spain is one of the countries with a low endemicity of hepatitis B, presenting a cumulative prevalence of HB surface antigen (HBsAg) carriers of 0.8% [13]. In 2016, there were 530 cases of hepatitis B in Spain and the resulting incidence was 1.14 cases per 100,000 inhabitants. Due to its chronic nature, hepatitis B represents a major public health problem, not only because of its associated morbidity and mortality but also and fundamentally because HBsAg carriers constitute the main reservoir of the virus.

Although the vaccine is effective in 95% of individuals, no studies have been conducted to determine whether it remains effective after 10 years or if a booster is required. An American research team monitored 1578 individuals in Alaska (United States) who

received three doses of the hepatitis B vaccine between 1981 and 1982 [14]. Fifteen years after vaccination, the team tested all subjects for antibodies that would protect them from infection and found that the number of antibodies against the virus had fallen since vaccination. The factors associated with a greater likelihood of continuing to present high antibody levels at 15 years were: being male, being older at the time of vaccination and having developed a high number of antibodies at the start [8–10,14–24].

In this regard, the study by Bruce et al. [17] provides widespread evidence that humoral immunity persists for ≥ 30 years, however, it requires studies to determine the state of cell-mediated immunity would be complementary and important, especially in those who lose anti-HBs and do not respond to a booster dose.

Although any individual without immunity to the hepatitis B virus (HBV) is susceptible to the disease, some groups are at greater risk due to high-risk practices, illness or simply personal contact. Health care staff comprise one of these groups, with a 2–10 times higher risk of infection than the general population. Transmission generally occurs through accidental exposure to patients' blood, primarily as a result of parenteral procedures [3,11,18,20,25].

Since 5% of the general population does not respond to the conventional vaccine, and because antibody levels decrease over time, seroprotection against the virus remains uncertain. Therefore, it is necessary to find an alternative to protect health service staff who do not have antibodies, either because they never generated a response or because they no longer respond to the conventional vaccine. Fendrix[®], the hepatitis B vaccine formulated with the new AS04 adjuvant (MPL + aluminum salts) by GlaxoSmithKline, may provide a good alternative [19,26–28]. The vaccine is indicated for patients with renal insufficiency, especially those on hemodialysis and pre-hemodialysis aged over 15 years old [19,22,28–35]. It has been reported that revaccination with Fendrix[®] has a high success rate in HIV patients who do not respond to other vaccination strategies against hepatitis B [30,34–35].

Compared with the conventional vaccine, the additional costs of administering Fendrix[®] to health service staff as a prophylactic against hepatitis B are more than justified if we consider the mean costs entailed in monitoring and treating hepatitis B in health service staff infected through accidental exposure [36,37], combined with the associated sick leave [38–39]. In addition, in the case of accidental infection, the cost of a reported occupational accident would be lower in immunised than in non-immunised subjects [40].

1.2. Specific objectives and hypothesis

- Objectives
 - o To endow Spanish Health Service staff with additional protection against hepatitis B infection.
 - o To assess the efficacy of the adjuvanted vaccine in healthy subjects who do not respond to the conventional hepatitis B vaccine.
- Hypothesis
 - o Administration of the Fendrix[®] vaccine is a viable alternative for Spanish Health Service staff classified as “non-responders”.

2. Methods

2.1. Clinical study design

2.1.1. Description of the clinical study design

We conducted a non-randomised, multi-centre, phase 4 clinical trial with a single group, without masking (open-label), administering medication for off-label use in Spanish Health Service staff whose work exposed them to biological risk and who had been classified as non-responders to the conventional hepatitis B vaccine.

2.1.2. Significant changes in the methods after initiating the trial

The main changes in this clinical trial concerned subject recruitment. As the initial rate of recruitment was lower than expected, we prolonged the recruitment time. This change modified overall study length and the dates of the trial stages. For similar reasons, we expanded our study population from the Autonomous Community of Castile-Leon Health Service (Spanish initials: SACYL) to the Spanish Health Service (Spanish initials: SNS) to enable inclusion of centres outside the Autonomous Community of Castile-Leon (Spanish initials: CyL). This led to the inclusion of 6 centres from the autonomous community of Madrid. Lastly, to further facilitate recruitment, the inclusion criteria were modified to include health sciences students undertaking a work placement with the SNS. This inclusion of students was subject to and limited by the specific regulations on health and safety in each autonomous region.

This clinical study was approved by the medical research ethics committee at the University Hospital of Salamanca on 25 September 2017, for implementation at the participating centres (ref. 17/1311), and was authorised by the Spanish Agency for Medicines and Health Products (Spanish initials: AEMPS) on 16 October 2017 (EudraCT number: 2016-004991-23) for implementation in these hospitals. (For protocol see <https://clinicaltrials.gov/ct2/show/NCT03410953>).

2.2. Participants

2.2.1. Participant selection criteria

In order to be recruited for this clinical trial, subjects had to work in the SNS—including university students undertaking work placements in SNS health centres (subject to and limited by the specific regulations on health and safety in each autonomous region)—and be of working age. In addition, they had to be free of any condition that contraindicated vaccination with Fendrix[®]. They also had to meet the criteria for classification as a non-responder to the conventional hepatitis B vaccine: anti-HBs antibody titers of <10 mIU/ml following administration of six 20 µg doses of conventional vaccine (two complete series). All subjects signed an informed consent form.

The exclusion criteria were as follows: known allergy to the active ingredient or to any other of the drug components (included in section 6 of the summary of product characteristics); a past

allergic reaction to any hepatitis B vaccine; or presenting a serious infection with fever at the time of recruitment. Subjects who did not give their informed consent were also excluded.

2.2.2. Site (centres and institutions) where data were collected

Eleven SNS hospitals participated in this clinical trial, and these are listed below:

- Complejo Asistencial Universitario de Salamanca. (Coordinating Center)
- Hospital Clínico Universitario de Valladolid
- Complejo Asistencial de Zamora
- Complejo Asistencial Universitario de León
- Complejo Asistencial Universitario de Palencia
- Instituto de Investigación Sanitaria Fundación Jiménez Díaz, Madrid.
- Hospital Clínico San Carlos, Madrid.
- Hospital Universitario 12 de Octubre, Madrid.
- Hospital Universitario Gregorio Marañón, Madrid.
- Hospital Universitario Puerta de Hierro, Madrid.
- Hospital Universitario Ramón y Cajal, Madrid.

2.3. Interventions

Fendrix[®] is a hepatitis B vaccine that contains the HBsAg adjuvant (3-O-desacyl-4'-monophosphoryl lipid A and aluminum phosphate). This adjuvant system is known as AS04 and it boosts vaccine immunogenicity. An anti-HB antibody titre of ≥ 10 mIU/ml correlates with protection against HBV infection.

Immunization consisted of a maximum of four 0.5 ml doses of Fendrix[®] administered as follows: 1, 2 and 6 months after the date of the first dose, by intramuscular injection of a 20 µg dose in the deltoid muscle, as indicated in the Fendrix[®] summary of product characteristics. We conducted a serological analysis about 30 days after administration of each dose. If subjects presented seroprotection (anti-HB ≥ 10 mIU/ml), administration of subsequent doses was suspended.

The Fendrix[®] vaccine lots used in this trial were: AFENA027BC (01/2020), AFENA028AI (05/2020) and AFENA029AC (09/2020).

We observed precautionary measures as regards the product contraindications and warnings indicated by the manufacturer and AEMPS recommendations for off-label use.

Thirty days after administration of each vaccine dose, we took a venous blood sample to determine anti-HBs titres. Serum samples were analysed immediately in the certified reference laboratory at each participating hospital, extracting the sample according to each laboratory's technical instructions. Anti-HBs values above 1000 mIU/ml were assigned a value of 1000 mIU/ml. The lower limit of detection for the trial was 3 mIU/ml. All values below the lower limit of detection were assigned a value of 3 mIU/ml.

Adverse effects were recorded during vaccine administration, noting all those that could be related to the vaccine and had appeared in the 30 days following each dose.

2.3.1. Results

After measuring the anti-HBs antibody titre before the first dose and one month after administration of each dose, the results for anti-HBs antibody titres were analysed to determine immune response to the adjuvanted vaccine. Anti-HBs titres greater than or equal to 10 mIU/ml were classified as positive.

To assess results for adjuvanted vaccine safety and clinical tolerance, adverse effects were recorded during administration, noting all those that could be related to the vaccine and had appeared within 30 days after each dose.

As secondary objectives, we analysed biochemical data collected immediately prior to administering the first vaccine to

determine participant characteristics. We also assessed the number of doses necessary to achieve a positive response. In addition, we analysed the influence of sex and age on the results.

2.3.2. Sample size

The total number of staff at the 11 centres participating in this clinical trial was approximately 47,650 at the time of conducting the trial. In an analysis that included 2620 eligible adults who had participated in 11 studies conducted in 10 countries, Van Der Meeren [41] described a 94.5% response rate to the conventional vaccine. Consequently, we estimated a target population for our trial of 2621 subjects. Raheja and colleagues [42] have indicated that for various reasons, about one-third of workers will refuse to participate in this type of trial, which would reduce our target population to 1730.

A literature review indicated that the mean percentage of workers who do not respond to the Fendrix[®] vaccine ranges from below 1% to 10% [7,15,19,29,32,35]. This would noticeably affect sample size calculation. Taking 5% as the mean percentage of workers who do not respond to Fendrix[®] and assuming an error margin of $\pm 1\%$, we required 70 subjects in total, with a range of 57–83, in order to achieve a 95% confidence level with a maximum acceptable error margin of 5%.

2.3.3. Randomization and masking

This was an open clinical trial without control group or masking.

2.3.4. Statistical analysis

We used two statistical methods to compare groups:

When both the variable of interest and the secondary variables were qualitative, we entered them into contingency tables and used Fisher's exact test to determine any dependency between both variables.

When the variable of interest was numeric and the secondary variables were qualitative, we used the Wilcoxon rank-sum test, since numeric variables do not generally come from normally distributed populations and therefore the classic parametric tests are not recommended. We used the Shapiro-Wilks test to determine normality, due to its higher power compared to other similar tests [43].

In all analyses, we eliminated missing values.

To describe the qualitative variables, we used frequency tables and bar graphs, obtaining confidence intervals for the first category of each variable.

Means and standard deviations were used to describe numeric variables, but also quartiles, due to the absence of normality, both globally and by category for some qualitative variables. We also estimated confidence intervals for the population mean of each variable, assuming unknown variance.

In addition, we calculated Spearman correlations for numeric variables to determine dependency between them. Once again, a non-parametric method was used due to the absence of normality.

3. Results

3.1. Participant flow chart

3.1.1. Recruitment

3.1.1.1. Dates that define the periods of recruitment and follow-up. From 26 April 2018, the date of the inclusion of the first subject, to 11 June 2019, the date of the inclusion of the last subject, 94 individuals were assessed for eligibility, of whom 67 were included in the present study (Fig. 1). Follow-up continued until 31 October 2019.

On 14 November 2019, the trial was concluded with 67 recruited subjects. Of the 94 subjects assessed for eligibility, 27 did not agree to participate in the trial and declined to sign an informed consent form.

In the absence of losses or exclusions, data analysis was conducted with 67 subjects.

3.1.2. Baseline data

3.1.2.1. Baseline demographic and clinical characteristics. The possible factors that could alter our primary result (e.g. age, baseline anti-HBs titre, diabetes, body mass index) were in line with those of a normal healthy population of non-responders to the conventional vaccine (Table 1, Table Anx1 and Table Anx2), with the exception of sex, whereby the population presented a marked shift towards women, reflecting the current composition of the health worker population in the Spanish Health Service.

3.1.3. Numbers analysed

As described in the interventions section, the vaccine was only administered to subjects whose previous serology was negative. Thus, the first dose was administered to 67 subjects, the second dose to 21 subjects, the third to 9 subjects and the fourth to 7 subjects.

3.1.3.1. Distribution of numeric variables. We used the Shapiro-Wilks test [43] to determine whether any of the numeric variables comes from a normal distribution (Gaussian). The hypotheses were:

- H_0 : the sample variable comes from a normally distributed population
- H_1 : the sample variable does not come from a normally distributed population

Most of the numeric variables (except "height" and "creatinine") did not present a normal distribution, with a significance of $\leq 5e-02$ (0.05) (see Table 2).

3.1.4. Results and estimation

Of the 67 subjects participating in this trial, 63 (94.03%) attained anti-HBs levels of ≥ 10 mIU/ml, indicating seroprotection against HBV (Table 3).

A cumulative analysis of the response data indicated a gradual increase in subjects reaching seroprotection: 68.66% with the first dose, 86.57% with the second dose, 89.55% with the third dose and 94.03% with the fourth dose (Fig. 2).

We did not detect any serious adverse effects during this clinical trial. Most of the adverse effects observed occurred after administering the first dose. Some 30% of vaccinations (32 out of 104) gave rise to adverse effects, and of these, 78.12% were related to the medication under study (Table 4). The symptoms were transient and almost all resolved spontaneously within 1 week.

By symptom, the most common effect (59.38%) was pain at the injection site. Seven adverse effects were not associated with the medication, and the most common of these was "malaise". Table 4 gives all the data obtained on adverse effects.

3.1.5. Secondary analysis

We found a relationship between sex and response to the first vaccine dose. This was determined by performing the Wilcoxon rank-sum test with continuity correction, where the hypotheses to test were H_0 : Anti-HBs (men) \geq Anti-HBs (women) and H_1 : Anti-HBs (men) $<$ Anti-HBs (women). The test yielded a p-value of 0.037 ($W = 318.5$), which is lower than 0.05, thus H_0 is rejected and H_1 accepted, indicating that men presented a lower level of Anti-HBs (Fig. 3).

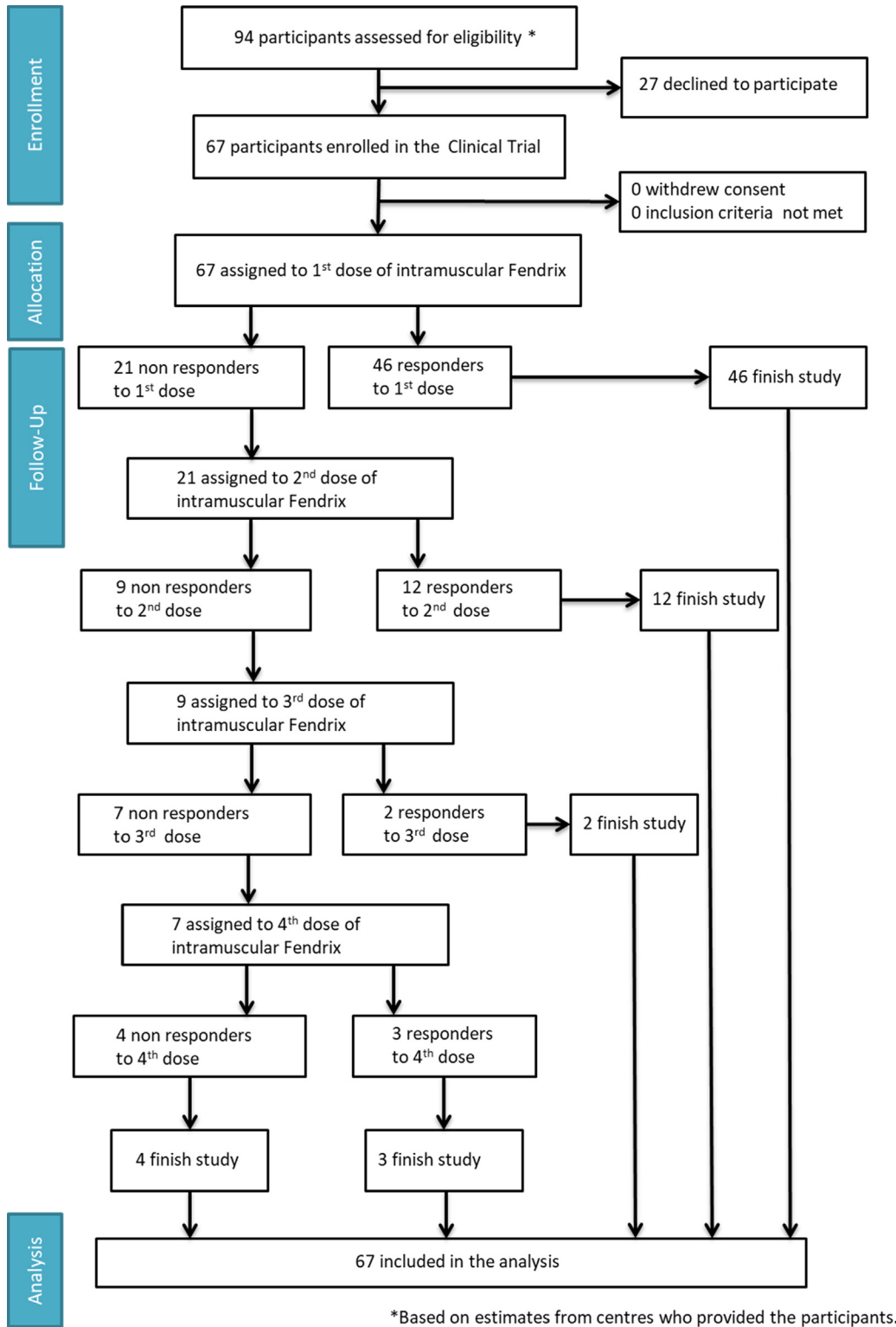


Fig. 1. Flow chart.

This effect was not observed for the following doses administered, probably because of the low “n” when these doses were administered. This effect has been described previously for other hepatitis B vaccines. [20,44]

Table 5 shows the quantitative variables that present significant differences by the sex categories. In all the variables, except

“AntiHbS visit 2”, the values in male are significantly higher than in female.

3.1.5.1. Spearman correlation matrix. We found the following significant correlations (p-value ≤ 0.05) as shown in Fig. Anx1:

- Age and Glucose, Urea, GGT, Visit_2_AntiHbS.

Table 1
Demographic and clinical baseline characteristics.

Age group	Number of subjects		Total
	Male	Female	
18–40 years	3	11	14
40–80 years	17	36	53
Global	20	47	67

	mean	sd	IQR	25%	50%	75%	n	NA
Age	49,46	11,77	14	43	53	57	67	0
Female	47,95	11,43	11,00	42	52	55	47	0
Male	53,00	12,07	20,75	44	58	62	20	0
Weight (kg)	71,67	15,73	21,25	59,3	70,0	80,5	66	1
Female	65,90	11,99	15,12	56,6	63,5	71,8	46	1
Male	84,93	14,48	16,27	74,1	80,0	90,3	20	0
Height (cm)	166,72	8,17	9,50	161,50	166,00	171,00	67	0
Female	162,70	5,12	6,50	160	163	166	47	0
Male	176,15	5,91	10,00	171	177	181	20	0

	Glucose		Urea		Uric Ac.		ALT-GPT		AST-GOT		GGT		Bilirubin		Creatinine		Anti HbS	
	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n
Normal	92,64	62	95,31	61	88,06	59	95,38	62	96,72	59	87,88	58	93,85	61	92,54	62	0	0
Abnormal	7,46	5	4,69	3	11,94	8	4,62	3	3,28	2	12,12	8	6,15	4	7,46	5	100	67

Table 2
Distribution of the numerical variables analyzed.

	Shapiro_Wilks_p.value
Age	7.851978e-05
Weight (Kg)	1.375092e-03
Height (cm)	6.245423e-02
Selection Visit AntiHbS	2.611177e-15
Selection Visit Glucose	1.172472e-02
Selection Visit Uric Acid	3.507400e-02
Selection Visit Urea	1.281404e-02
Selection Visit Creatinine	1.598279e-01
Selection Visit Bilirubin	8.114063e-10
Selection Visit AST_GOT	4.851082e-11
Selection Visit ALT_GPT	1.844211e-11
Selection Visit GGT	4.085315e-13
Visit 2_AntiHbS	2.638358e-11
Visit 3_AntiHbS	6.580777e-06
Visit 4_Serolog_AntiHbS	7.054380e-05
Final visit AntiHbS	1.000965e-05

Consequently, we used non-parametric tests with these variables to test other associated hypotheses.

- Weight and Height, Selec_visit_AntiHbS, Glucose, Uric acid, Creatinine, ALT_GPT, GGT.
- Height and Uric acid, Creatinine, Bilirubin.
- Glucose and Uric acid, GGT.
- Uric Acid and ALT_GPT, GGT.
- Urea and Creatinine.
- Creatinine and GGT
- AST_GOT and ALT_GPT.
- ALT_GPT and GGT, Visit_2_AntiHbS.
- GGT and Visit_2_AntiHbS.
- Visit_3_AntiHbS and Visit_4_AntiHbS.

Table 3
Vaccination response by dose.

Dose	Vaccinated	Responders	Non-responders	% response
First	67	46	21	68,66%
Second	21	12	9	57,14%
Third	9	2	7	22,22%
Fourth	7	3	4	42,86%
Total	67	63	4	94,03%

We also analysed the influence of age on vaccine response and observed a significant correlation ($r = -0.37$ with p -value = 0.0019) between age and response to the first dose. This finding indicates an inverse correlation between variables, whereby the higher the age, the lower the response (Fig. 4). This effect was not observed for the following doses administered, probably because of the low “n” when these doses were administered.

3.1.5.2. Contingency tables and test of independence. To study dependence between two qualitative variables, we performed Fisher’s exact test, where the hypotheses were:

- o H_0 : Variables are independent
- o H_1 : Variables are dependent

The most relevant pair of variables that yielded a significant p -value was:

- “Anti-HBs visit 2” and “Adverse effects visit 2”, p -value = 0.0005149.

This indicates that the adverse effects reported following administration of the first dose appear to be linked to not attaining seroprotection (Fig. 5). This effect was not observed for the following doses administered, probably because of the low “n” when these doses were administered.

No relationship was found between the biochemical parameters analysed –glucose, uric acid, urea, creatinine, bilirubin, AST-GOT, ALT-GPT and GGT– and the response to vaccination with Fendrix®.

3.1.5.3. Influence of the time between the last dose of conventional vaccine and revaccination with Fendrix on the response to vaccination. We analyzed the influence of the time elapsed between pre-

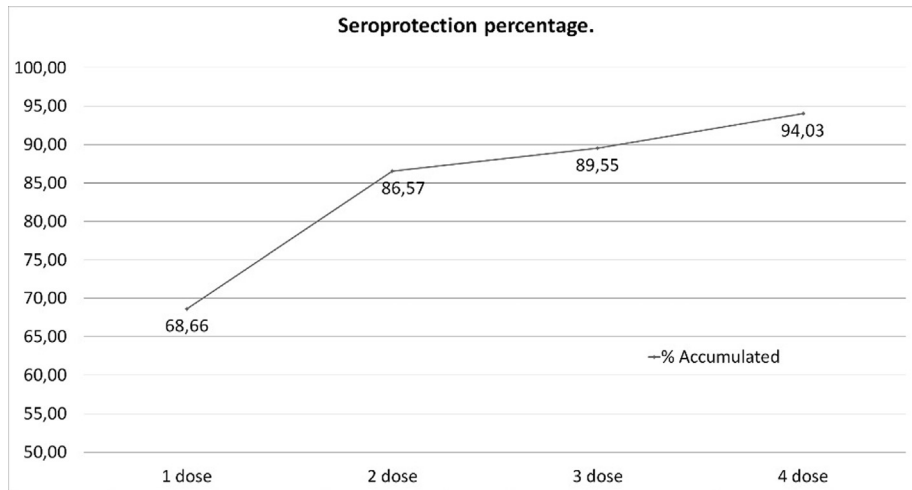


Fig. 2. Cumulative response to vaccination. Cumulative percentage of individuals responding to the vaccine after each dose. After the first and second doses, a very high percentage of positive response to the vaccine is achieved.

Table 4
Adverse Events (AE) found during the trial.

	Dose 1		Dose 2		Dose 3		Dose 4		Total n	Overall %
	%	n	%	n	%	n	%	n		
Subjects with AE	31,30	21	28,57	6	11,11	1	14,28	1	32	
Detected AE		24		6		1		1		
Related to Medication										
Yes	76,20	19	83,33	5	100,00	1	0,00	0	25	78,12
No	23,80	5	13,66	1	0,00	0	100,00	1	7	21,87
Type										
Puncture Zone Pain	58,33	14	66,67	4	100,00	1	0,00	0	19	59,38
Discomfort	12,50	3	16,67	1	0,00	0	0,00	0	4	12,50
Fatigue	12,50	3	0,00	0	0,00	0	0,00	0	3	9,38
Diarrhea	8,33	2	0,00	0	0,00	0	0,00	0	2	6,25
Paresthesia in the puncture area	8,33	2	0,00	0	0,00	0	0,00	0	2	6,25
Cramp	0,00	0	16,67	1	0,00	0	0,00	0	1	3,13
Diverticulitis	0,00	0	0,00	0	0,00	0	100,00	1	1	3,13

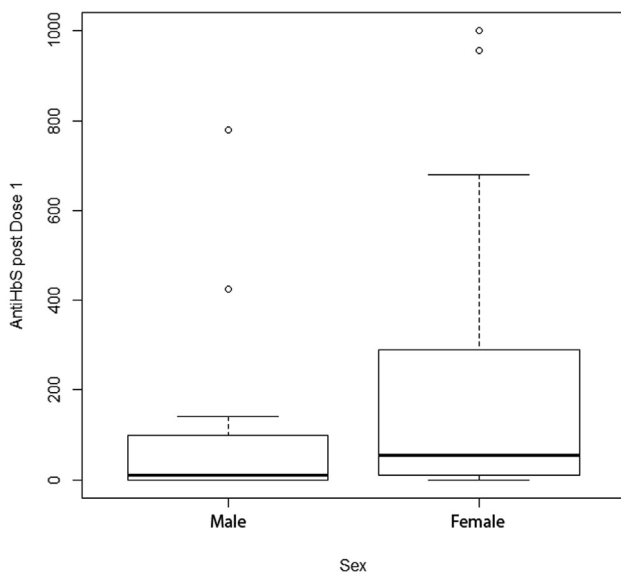


Fig. 3. Response to the first dose of vaccine; comparative by sex. Boxplot of the distributions by sex of 'AntiHbS after dose 1' where we observe that males present lower values and with less dispersion than females.

Table 5
Wilcoxon test of the quantitative variables that present significant differences by sex.

	Sex	
	W	p-value
Age	627	3,10E-02
Weight (kg)	789,5	4,39E-06
Height (Cm)	909,5	1,71E-09
Uric acid (mg/dL)	751	1,20E-04
Urea (mg/dL)	521	2,40E-02
Creatinine (mg/dL)	874	3,19E-08
Bilirubin (mg/dL)	620,5	1,50E-02
AST-GOT (U/L)	566	1,60E-02
ALT-GPT (U/L)	649	4,70E-03
GGT (U/L)	690,5	1,30E-03
AntiHbS Visit 2 (mUI/ml)	318,5	3,70E-02

study vaccination with the conventional vaccine and vaccination with Fendrix for the trial. We do not see any relationship between the time between vaccines and the response to vaccination with Fendrix. Analyzing the final response to Fendrix, we find two very unbalanced groups, to which after applying “Wilcoxon rank sum test with continuity correction” we obtain $W = 144$ and p -value

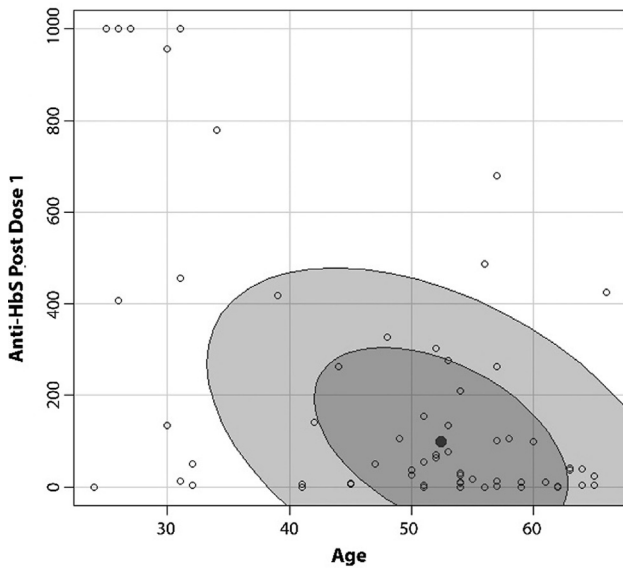


Fig. 4. Response to the first dose of vaccine; Influence of age. Scatterplot between 'age' and 'AntiHbS after dose 1' showing that low levels of AntiHbS are mainly concentrated in individuals aged between 50 and 60 years.

= 0.6003 showing no significant differences between both groups (Fig. Anx2).

Analyzing the initial response to Fendrix, we find two more balanced groups, to which after applying “Wilcoxon rank sum exact test” we obtain $W = 540$ and $p\text{-value} = 0.3592$, not showing significant differences between the two groups (Fig. Anx3).

3.1.5.4. Dependence between response and other factors. Finally, we analyzed whether any immunosuppressive conditions, such as the use of steroids or immunosuppressive diseases, or whether previous or current pathologies, or if the concomitant medication in the study had any influence on the response to revaccination with Fendrix. These data were analyzed with the total response to vaccination and the response to the first dose of Fendrix.

The only relevant data resulting from the analysis carried out is that there is a dependency between “Global response” and “Possible conditioning pathology -YES/NO-” ($p\text{-value} = 0.0063$) (Fig. Anx4) and between 'Global response' and “Concomitant medication -YES/NO- ”($p\text{-value} = 0.0092$) (Fig. Anx5).

There are insufficient data to determine whether these associations are due to a specific pathology or to a specific drug.

3.1.6. Adverse effects (risk)

The 67 participants reported 32 adverse effects (AEs), of which 25 were adverse reaction (AR). None of the adverse effects reported were considered serious and all patients recovered from their corresponding adverse effect, suggesting a low risk of serious adverse effects and an acceptable risk in relation to non-serious adverse effects, which corresponded to those already identified in the Technical Data Sheet.

Four subjects had diabetes mellitus type 2 prior to participating in the trial. Van Der Meeren et al. [45] reported that the reactogenicity and safety profile of the hepatitis B vaccine (Engerix-B™) appeared similar in controls and patients with hepatitis B and was consistent with the experience of the vaccine). Two of them did not reach a response after 4 doses of Fendrix® and the others attained seroprotection after the fourth dose. Another non-responder had autoimmune hepatitis prior to participating in the trial and the last non-responder did not present any pathologies of interest.

3.1.6.1. Risk-benefit assessment. An analysis of the results revealed no evidence of significant, identifiable or potential risks related to administration of Fendrix® in the study population (health service staff and university students whose work in the SNS exposed them to biological risk and who had been classified as non-responders to the hepatitis B vaccine), beyond those already indicated in the Technical Data Sheet.

3.1.7. Discussion

3.1.7.1. Limitations. Conducting the clinical trial in accordance with the instructions of the Spanish Agency for Medicines and Health Products (AEMPS) generated a slight difficulty. The AEMPS has ruled that Fendrix® should be administered as described in the summary of product characteristics. Our trial observed these spec-

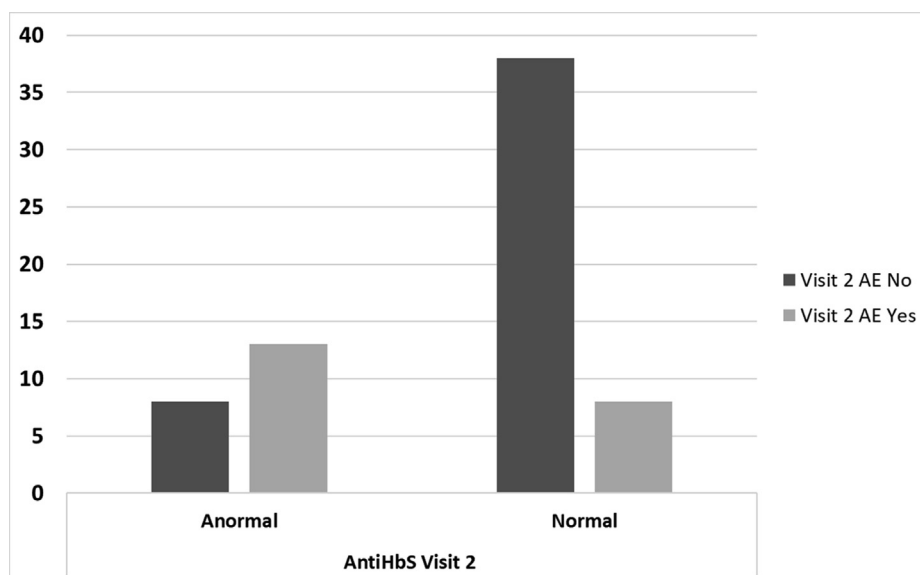


Fig. 5. Relationship between the presence of Adverse Events after the first dose administered and acquisition of seroprotection (≥ 10 mIU/ml). Barplot between 'AntiHbS after dose 1' and 'Presence of adverse events' showing that individuals with abnormal values of 'AntiHbS after dose 1' had adverse events.

ifications, which required an effort on the part of all those involved. However, this limitation endowed our trial with added value.

One of the greatest difficulties we encountered while conducting this trial was recruitment, as samples from all hospitals were small. This resulted in the need to extend recruitment to additional centres in order to obtain an optimal study sample.

We also encountered a lack of information concerning staff who do not respond to vaccination. The exclusion of the Fendrix[®] vaccine for off-label use, or recent approval for use in patients with immunodeficiency, diverts attention away from non-responder staff. Consequently, treatment with specific immunoglobulin remains the only possible option to treat a worker potentially infected with the hepatitis B virus, despite the existence of a much better alternative: the Fendrix[®] vaccine.

Lastly, we also encountered some isolated difficulties as regards organizing all trial visits and their dates for various reasons, including vacations and sick leave. Although this posed an added difficulty, it was resolved through study coordination.

3.1.7.2. Extrapolation. The off-label use of Fendrix[®] described here will enable its administration to many potential beneficiaries if the AEMPS approves such off-label use. These include:

- o Health service staff
- o Non-health service staff
- o Employees in workplaces related to health care
- o State security forces
- o Prison officers

Our results pave the way for replicating the trial in other countries.

3.1.7.3. Interpretation. From the perspective of health, Fendrix[®] represents a viable alternative vaccine for SNS staff classified as “non-responders”.

As regards costs, according to previous publications, the “cost” of the vaccine is offset by savings in:

- The cost of follow-up after a biological accident.
- The cost of administering specific immunoglobulin.
- The personal cost to the worker concerned.
- The level of response to a single dose.

Other information.

Trial registration: The trial was registered in the Spanish National Trial Register (REEC), ClinicalTrials.gov and inclusion has been stopped (identifier NCT03410953; EudraCT-number 2016–004991–23).

Protocol: For protocol see <https://clinicaltrials.gov/ct2/show/NCT03410953>

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Ethics approval and consent to participate: All procedures in the study were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical

standards. Informed consent was obtained from all individual participants included in the study.

CRediT authorship contribution statement

José L. Bravo-Grande: Conceptualization, Methodology, Supervision, Funding acquisition, Writing - original draft. **M. Asunción Blanco-González:** Investigation, Resources. **José M. de la Torre-Robles:** Investigation, Resources. **Marita P. Asmat-Inostrosa:** Investigation, Resources. **Marina Fernández-Escribano:** Investigation, Resources. **Itria Mirabella Villalobos:** Investigation, Resources. **M. Covadonga Caso-Pita:** Investigation, Resources. **Marina Hervella-Ordoñez:** Investigation, Resources. **Luis M. Cañibano Cimas:** Investigation, Resources. **José M. de la Fuente-Martín:** Investigation, Resources. **M. Luisa Rodríguez de la Pinta:** Investigation, Resources. **Jenry Ricardo Borda Olivás:** Investigation, Resources. **Carmen Muñoz-Ruipérez:** Investigation, Resources. **Miguel A. Alonso López:** Investigation, Resources. **Teresa del Campo-Balsa:** Investigation, Resources. **M. Antonieta Ramírez Pérez:** Investigation, Resources. **Ignacio Sánchez-Arcilla:** Investigation, Resources. **Marco Marzola-Payares:** Investigation, Resources. **Fernando Rescalvo-Santiago:** Investigation, Resources. **Miguel de Paula-Ortiz:** Investigation, Resources. **José M. Sánchez-Santos:** Formal analysis, Data curation, Visualization. **Ricardo López-Pérez:** Methodology, Validation, Data curation, Project administration, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2020.12.006>.

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