Ethnicity and response to primary three-dose hepatitis B vaccination in employees in the Netherlands, 1983 through 2017

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Abstract

Background & Aims

Hepatitis B virus (HBV) vaccination is recommended to all employees who have an occupational risk in the Netherlands. This study assessed the determinants of immune response to primary standard 3-dose HBV vaccination (0, 1, 6 months), with the main focus on ethnicity.

Methods

Out of 76,239 individuals who received HBV vaccination between April 1983 and December 2017, 11,567 persons with known country of birth and complete vaccination schedule were included in this study. Weighted multiple logistic regression with Firth's bias adjustment was used to assess determinants of non-response (anti-HBs < 10 mIU/mL) and low-response (anti-HBs 10 – 99 mIU/mL).

Results

Baseline characteristics of the study population (n = 11,567) were as follows: mean age 27.5 years (95% CI 27.23 – 27.72), 99.4% born in the Netherlands and 93.5% of Western European origin. Of all identified subjects, 180 (1.6%) were HBV vaccine non-responders and 549 (4.8%) were low-responders. When compared to individuals

aged <40 years, the rate of non-response (4.3% vs 0.8%, $p < .001$) and low-response (11.9% vs 2.9%, $p < .001$) was higher in those aged 40 years or older. The height of anti-HBs levels were lower in those subjects aged > 40 years in comparison to those younger than 40 years, $p < .001$ All non-responders were born in the Netherlands. Although no significant association was found between non-response and individuals of Western European origin (adjusted odds ratio (aOR)=1.20, 95% CI 0.66 – 2.44, $p = .163$), low-response to HBV vaccination was significantly associated with Western European origin (aOR=2.21, 95% CI 1.41 – 3.86, $p = .001$). Significant determinants for non-response were older age at vaccination (aOR=1.06, 95% CI 1.06 – 1.07, $p < .001$) and male gender (aOR=2.51, 95% CI 1.97 – 3.22, $p < .001$).

Conclusions

The non-response rate was low in our study population. Our findings suggest that the vaccines being used for the primary vaccination are probably less immunogenic for older individuals, males and persons of Western European origin.

Abstract word count: 246

Keywords: Hepatitis B; Health-care workers; Ethnicity; non-response; determinant.

Introduction

Hepatitis B virus (HBV) is still a constant and serious threat to world health. Each year, around 700,000 HBV-related deaths occur worldwide, making it together with hepatitis C virus infection the 7th leading cause of mortality globally in 2013.[1] The occupational risk of HBV infection in health-care workers has long been recognized. The virus remains infectious for prolonged periods on surfaces and is transmissible even in the absence of visible blood.[2] In serological studies conducted in Europe,

health-care workers had a greater risk of HBV infection than the general population.[3, 4] The risk of acquisition of HBV infection in a nonimmune person after a needlestick exposure is at least 30% if the patient is hepatitis B e antigen (HBeAg) positive and 6% if the patient is HBeAg negative.[5, 6]

Hepatitis B vaccines to prevent HBV infection and its complications became available in Europe in 1981, and were recommended by the Advisory Committee on Immunization Practices (ACIP) for health-care workers in 1982.[7] An estimated 95% of healthy adults develop protective levels of antibodies against hepatitis B surface antigen (anti-HBs > 10 mIU/mL) after primary HBV vaccination.[8] A lower response to vaccination has been associated with age above 40 years, smoking, obesity, male gender and immunodeficiency.[9] Persons with this deficient antibody response (anti-HBs < 10 mIU/mL) are called non-responders and are thought to remain susceptible for HBV infection. Yet, there is no research exploring the effects of ethnicity on the immune response to a primary hepatitis B vaccination schedule in the healthy adult population in Western Europe.

A study on 202 twin pairs by Hohler et al[12] indicated that human histocompatibility leucocyte antigen (HLA) genes might account for the majority of immune responsiveness to hepatitis B. Several genetic studies have further demonstrated considerable differences in HLA DQ2 phenotype, which has been associated with poor- and non-response to hepatitis B vaccination, with higher frequency in Western Europe (5-20%) in comparison to 5-10% in North Africa, the Middle East and China.[13-17] With the growing cultural diversity in Western Europe, the present study assessed the influence of ethnicity on response to primary standard three-dose HBV

vaccination in employees in the Netherlands. We hypothesized that the Western population was responsible for poorer response to primary hepatitis B vaccination.

Patients and methods

Participants

For this retrospective study, we analyzed data from the VAXIN medical records of Ease Travel Clinic & Health Support, situated in the Limburg province of the Netherlands with a population of 1,117,198. Ease Travel Clinic & Health Support is specialized in vaccinations of individuals at occupational risk and the VAXIN medical records include data on ethnicity, date of birth, gender, type of vaccination, date of first, second and third hepatitis B vaccination, time of anti-HBs level determination and height of anti-HBs level. Students and employees with an occupational risk of HBV infection (e.g. in health care, police, fire department), 16 years of age and older who received primary HBV vaccination between 27 April 1983 and 20 December 2017 were included in the study. All subjects were born before the start of the implementation of hepatitis B vaccination in the Dutch immunization program (1 Augustus 2011). Subjects with unknown country of birth, unknown gender, incomplete vaccination data and inadequate post-vaccination anti-HBs level analysis (i.e. post-vaccination anti-HBs level analysis more than 6 months after the last vaccination) were excluded. Figure 1 shows the flowchart of the study.

The study was approved by the ethics committee of Maastricht University Medical Center. Following Dutch regulation, the need of informed consent was waived seen the non-interventional character of our study. Data lock was on 6 May 2018.

Vaccines

The hepatitis B vaccination series for adults consisted of three vaccines, at 0 – 1 – 6 months. From 27 April 1983 to 31 December 1987, individuals received the plasma vaccine HBVax© 20 microgram/mL (Merck Sharp & Dohme, West Point, USA). Between 1 January 1988 and 31 December 2017, the commercially available recombinant Engerix-B© 20 microgram/mL (GlaxoSmithKline, Zeist, the Netherlands) was given. According to the local guidelines, the vaccines were injected into the deltoid muscle of the left or right arm with adequate 23-gauge needle with a length of 25 mm. There was a quality system for vaccine logistics, adequate storage and quality controlled chain management.

Study outcomes

The primary objective of this retrospective study was to evaluate the influence of ethnicity and other determinants on non-response after primary standard three-dose hepatitis B vaccination in students and employees who have an occupational risk in the Netherlands. In agreement with the recommendations of the ACIP, non-response was defined as an anti-HBs level < 10 mIU/mL measured between 1 – 6 months after the third vaccination.[10] Secondary objectives were the influence of ethnicity and other determinants on low-response and height of anti-HBs level after the last vaccination. Low-response was defined as an anti-HBs level within 10 – 99 mIU/mL.

Laboratory methods

Serum samples were tested for quantitative anti-HBs levels using a routine microparticle enzyme immunoassay (Anti-HBs, AxSYM AUSAB, Abbott, USA) or chemiluminescence assay (Anti-HBs, Cobas 8000, Roche, Germany).

Statistical analysis

Continuous variables were expressed as mean (95% confidence interval (CI)). Since the proportions of male participants of Western and non-Western European origin were lower in the sample than in the general population, post-stratification weights were applied correcting for gender and origin (Western European origin vs non-Western European origin). The gender and origin distribution of inhabitants of Limburg, the Netherlands, was taken from the last Central Bureau Statistics of February 2018.[11] Associations between non-response/low-response and country of birth, origin, age at vaccination, gender, type of vaccination (plasma or recombinant vaccine), weeks between 1st and 2nd vaccination, weeks between 2nd and 3rd vaccination, and weeks between 3rd vaccination and anti-HBs level determination were assessed using weighted tests (ANOVA for continuous and chi squared for categorical variables). Weighted multiple logistic regression with Firth's bias adjustment was used to assess determinants of non- and low-response. Weighted multiple linear regression was used to assess determinants of GMT. Model selection was done in a backward stepwise manner. A sensitivity analysis was conducted excluding severe outliers (anti-HBs level > 1,000 mIU/mL) in order to investigate their influence on the results. All statistical analyses were performed using RStudio (Version 1.0.136, Boston, MA). The level of statistical significance was set at .05.

Results

Demographics

During the study period, 76,239 adult individuals received primary three hepatitis B vaccinations. After excluding individuals with unknown country of birth (n = 59,473), unknown gender (n = 3,706), incomplete vaccination data (n= 867), and post-vaccination anti-HBs level analysis > 6 months after the third vaccination (n = 626),

11,567 persons were identified according to in- and exclusion criteria (Figure 1; Table 1). Of all identified subjects, 180 (1.6%) were hepatitis B vaccine non-responders and 549 (4.8%) were low-responders.

Characteristics of the subjects excluded based on a delayed titre analysis (> 6 months after the third vaccination) were: mean age of 29.7 years (95% CI 28.45 – 30.92), 35.3% being male, 97.3% with Dutch country of birth, and 90.4% of Western European origin. This differed significantly from the characteristics of the original study population ($p < .001$, $p < .001$, $p < .001$ and $p = .005$, respectively).

HBV vaccine non-responders

A total of 180 (1.6%) adults had an anti-HBs level < 10 mIU/mL. Four (2.2%) of them were positive for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core antigen (anti-HBc). All of the non-responders were born in the Netherlands. The mean age of non-responders was 42.3 years (95% CI 39.87 – 44.64 years) and 42.2% were male. When compared to individuals aged <40 years, proportion of non-response was higher in those aged > 40 years (4.3% vs 0.8%, $p < .001$). A comparison of baseline characteristics between non-responders and responders is shown in Table 2. Factors significantly associated with non-response in weighted univariate analyses were age at vaccination ($p < .001$), male gender ($p < .001$), time between 2nd and 3rd vaccination ($p < .001$), and time between 3rd vaccination and anti-HBs level determination ($p < .001$).

Country of birth and type of vaccine were not included in further analyses because all non-responders were born in the Netherlands and all had received the recombinant vaccine. In the multiple logistic regression model, there was a higher odds of non-

response in individuals of Western European origin, although not significant (aOR=1.20, 95% CI 0.66 – 2.44, p = .163). Older age at vaccination (aOR=1.06, 95% CI 1.05 – 1.07, p < .001), male gender (aOR=2.39, 95% CI 1.81 – 3.23, p < .001), weeks between 1st and 2nd vaccination (aOR=1.00, 95% CI 0.99 – 1.004, p = .044), weeks between 2nd and 3rd vaccination (aOR = 1.01, 95% CI 1.003 – 1.01, p < .001), and weeks between 3rd vaccination and anti-HBs level assessment (aOR=1.10, 95% CI 1.07 – 1.13, p < .001) were found to be significant determinants of non-response. Based on predictions from this model, a non-response rate of 5% was found in males aged 43 years or over at the time of vaccination and females aged 58 years or over. At the ages at vaccination of 54 years in males and 73 years in females, the non-response rate increased to 10% in both groups (Figure 2).

HBV vaccine low-responders

HBV vaccine low-response occurred in 549 (4.8%) of all identified subjects (Table 3). The proportion of low-response was higher in individuals aged > 40 years vs < 40 years (11.9% vs 2.9%, p < .001). Country of birth and type of vaccine were not included in the weighted logistic regression models since none of the low-responders received the plasma vaccine and all but one were born in the Netherlands. Older age at vaccination (aOR=1.05, 95% CI 1.04 – 1.05, p < .001), male gender (aOR=1.71, 95% CI 1.44 – 2.03, p < .001), being of Western European origin (aOR=2.21, 95% CI 1.41 – 3.86, p =.001) and time between last vaccination and anti-HBs level assessment (aOR=1.09, 95% CI 1.07 – 1.11, p < .001) were significantly associated with low-response. Figure 3 shows the predicted low-response rates by age at first vaccination, for gender (A) and origin (B). A faster increase in low-response rate with

age was found in males compared to females and persons of Western European origin compared to those of non-Western European origin.

Height of anti-HBs level

In the total sample, mean anti-HBs level was 865.9 mIU/mL (95% CI 853.25 mIU/mL – 878.45 mIU/mL) (Table 4). Mean anti-HBs levels were 696.9 mIU/mL (95% CI 671.8 mIU/mL – 722.0 mIU/mL) and 912.8 mIU/mL (95% CI 898.4 mIU/mL – 927.2 mIU/mL) in those subjects aged > 40 years and those younger than 40 years, respectively, $p < .001$. Type of vaccine was not included in further analyses since there were only three included subjects who received the plasma vaccine. Older age at vaccination (b=-6.93, SE=0.46, $p < .001$), male gender (b=-74.57, SE=12.10, $p < .001$), and weeks between last vaccination and anti-HBs level assessment (b=-13.29, SE=1.76, $p < .001$) significantly decreased height of anti-HBs level.

Sensitivity analysis

The sensitivity analysis after removal of individuals with anti-HBs level > 1,000 mIU/mL showed that all baseline characteristics remained the same. Determinants for non- and low-response remained similar to the analyses including these outliers. For height of anti-HBs levels, some determinants were found in addition to those found in the previous analyses: being of Western European origin (b=-8.75, SE=2.78, $p = .001$) and weeks between 1st and 2nd vaccination (b=-0.31, SE=0.03, $p < .001$) significantly decreased height of anti-HBs level. However, the model without outliers for height of anti-HBs level did not meet the assumptions for multiple linear regression (e.g. normal distribution of residuals, homoscedasticity), whereas the model including

outliers did. These findings indicate that the discussion should be based on the results of the analysis in all identified subjects including those with higher anti-HBs levels.

Discussion

Ethnic differences in immune response to hepatitis B vaccinations might highlight the interplay between genetic and environmental factors. In this study, we found that 1.6% of the employees who have an occupational risk in the Netherlands were hepatitis B vaccine non-responders and 4.8% were low-responders. Ethnicity, i.e. being of Western European origin, was a significant determinant for low-response. However, the effect of ethnicity was not observed for non-response; this might be because the majority of our study population was of Western European origin (> 90%). To our knowledge, this is the first study assessing the role of ethnicity on immune response to primary HBV vaccination in a large group of healthy students and employees in Western Europe.

Although not assessing the influence of ethnicity after primary three vaccinations, non-response to booster hepatitis B vaccination in adults was higher in Taiwan's indigenous ethnicity than in Chinese individuals.[18] Phylogenetic studies have shown that Taiwanese people are descendants of Malay-Polynesian ethnicity and are genetically different from Chinese.[19, 20] In line with our findings, the influence of ethnicity was also observed in patients with end-stage renal disease, more specifically white race was a predictor of poor response to HBV vaccine.[21] On the other hand, previous studies in infants and children were not able to find a relation between ethnicity and hepatitis B vaccine non-response.[22-24] This might in part be explained by the (1) high effectiveness of recombinant hepatitis B vaccines in infants and children and (2) low number of subjects in the prior studies.

Other groups have demonstrated a non-response rate of 5 – 21%[8, 25-29] and a low-response rate of 24%[29] after primary HBV vaccination in health-care workers. This is in contrast to the findings of our present study and suggests that other study groups might have included more individuals with a higher chance of non-response. In the paper of Averhoff et al[8], 30% of the health-care workers were smokers which is higher than the 6 – 18% of most U.S. health-care workers.[30] In their study, 15% reported having a chronic disease and the mean age of health-care workers was 41 years.[8] The mean age of the included health-care workers was unknown in the study of Ciorlia et al[27] and 43 years in the study by Louther et al[26]. In our present study, students at occupational risk were included in addition to employees and with a mean age of 28 years, our study population was much younger. Older age is a well-known risk factor for a poor immune response and the gradual deterioration of the immune system already appears to occur at young age.[31]In that respect, our study confirmed the higher risk of non-response in males with a faster increase with age at the time of vaccination compared to females. A non-response rate of 5% was found in males aged > 43 years and females aged 58 years or over. Observations of lower anti-HBs response with aging have been linked to immunosenescence, i.e. the physiological deterioration of the immune system.[32] The effect of males might be owing to testosterone that damages the production of immunoglobulins from B-lymphocytes.[32]

Next to older age and male gender, longer time between 3rd vaccination and anti-HBs level determination was associated with non-response, low-response and decreased height of anti-HBs level in the present study. Likewise, time between 1st and 2nd vaccination and time between 2nd and 3rd vaccination were found to be significant determinants for non-response after controlling for other factors. In our study, we excluded employees who had a post-vaccination anti-HBs level analysis > 6 months

after the last vaccination in agreement with the recommendations of the ACIP.[10] This is important for a true estimation of non-response after primary HBV vaccination as the anti-HBs levels wane over time.[33, 34] Moreover, as illustrated in our study, even intervals 4 months between the final hepatitis B vaccination dose and postvaccination serologic testing have been associated with waning of anti-HBs levels in prior studies.[35, 36] Our finding of a lower immune response to primary hepatitis B vaccination with an extended interval between 1st and 2nd vaccination is consistent with previous studies.[37, 38] The reasons for this are uncertain, but it should be noted that in both our and previous studies differences were of borderline statistical significance.

There are some limitations to the present study. First, our study was limited by its inability to account for other risk factors, particularly immunodeficiency, smoking and Body Mass Index. Nevertheless, only a few people are expected to have an underlying immunodeficient condition in this apparently healthy group of students and employees. Moreover, several studies suggested that smoking and Body Mass Index were of less importance than age and gender in the immune response to hepatitis B vaccination.[31, 39] Second, the study population could be biased towards younger individuals, females, persons born in the Netherlands and individuals of Western European origin due to the stringent in- and exclusion criteria of the present study. Particularly, we found that subjects excluded based on the time of post-vaccination anti-HBs level assessment > 6 months after the third vaccination were older, more often males, less frequently born in the Netherlands and were less frequently of Western European origin. This exclusion of older individuals and males could have led to an underestimation of the non- and low-response rates in our study population, while the diminished representation of individuals of Western European

origin could have contributed to an overestimation of the proportion of non- and low-responders in the current study.

Third, students and employees are not routinely screened for HBsAg, anti-HBc, anti-HBs, antibodies against hepatitis C virus (anti-HCV), or antibodies against HIV (anti-HIV) before the initiation of primary hepatitis B vaccination in the Netherlands. The Netherlands is a low HBV endemic country with HBsAg and anti-HBc prevalence of 0.2% and 3.5%, respectively.[40, 41] Moreover, the universal hepatitis B vaccination in the Netherlands has been implemented just recently on 1 Augustus 2011. With a low prevalence of anti-HCV (0.7%) and anti-HIV (0.2%) in the Netherlands,[41, 42] we do not expect that the implementation of routine screening for hepatitis B, hepatitis C and HIV before the initiation of hepatitis B vaccination would have had a major impact on the current results. This is reinforced by the finding that only four of the non-responders in the current study were positive for HBsAg and anti-HBc. Fourth, although the height of anti-HBs antibody levels 1 – 6 months after the third vaccination has been associated with the persistence of anti-HBs and the incidence of reducing HBV infections,[33, 34] we did not measure these long-term results which might be important from a public health point of view.

In brief, it seems that non- and low-response rate is low among students and employees who have an occupational risk in the Netherlands. Our findings suggest that the vaccines being used for the primary vaccination are probably less immunogenic for older individuals, males and persons of Western European origin. More research is needed to investigate the effects of HLA alleles and other immune-related genes on the response to hepatitis B vaccination in persons of Western European origin.

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Figures

Fig. 1. Flowchart of the study

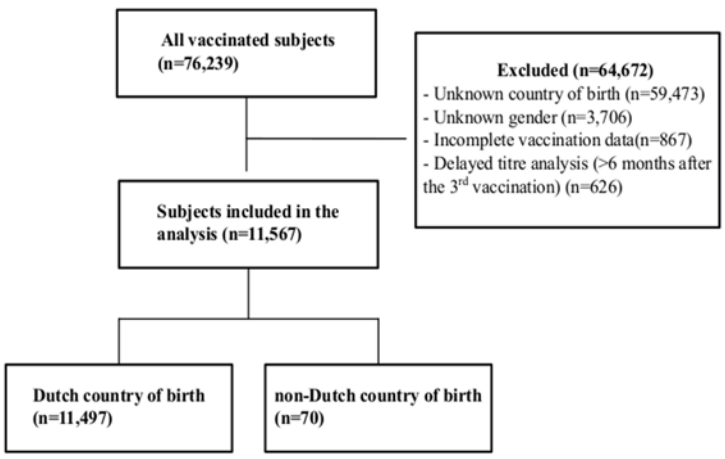


Fig. 2. Predicted non-response to hepatitis B vaccination over age at vaccination for males and females

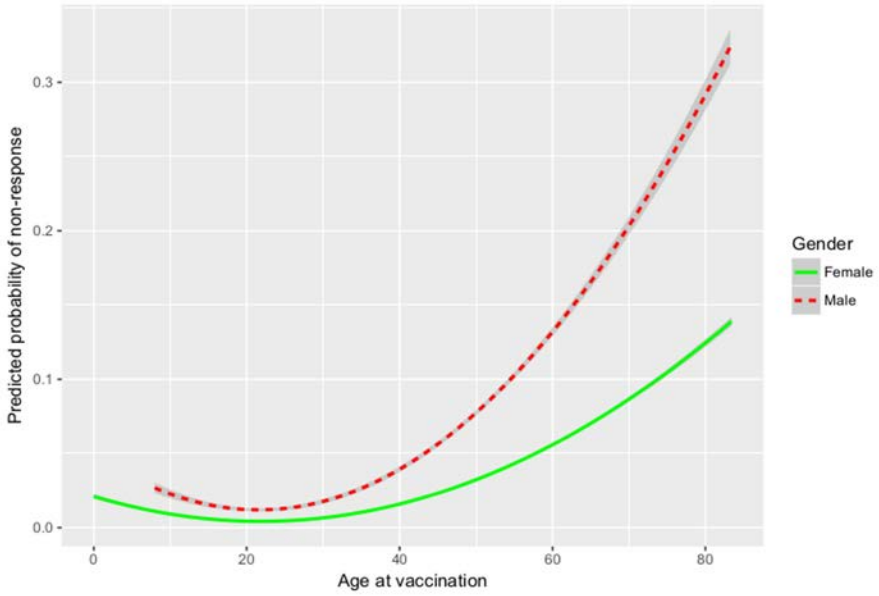
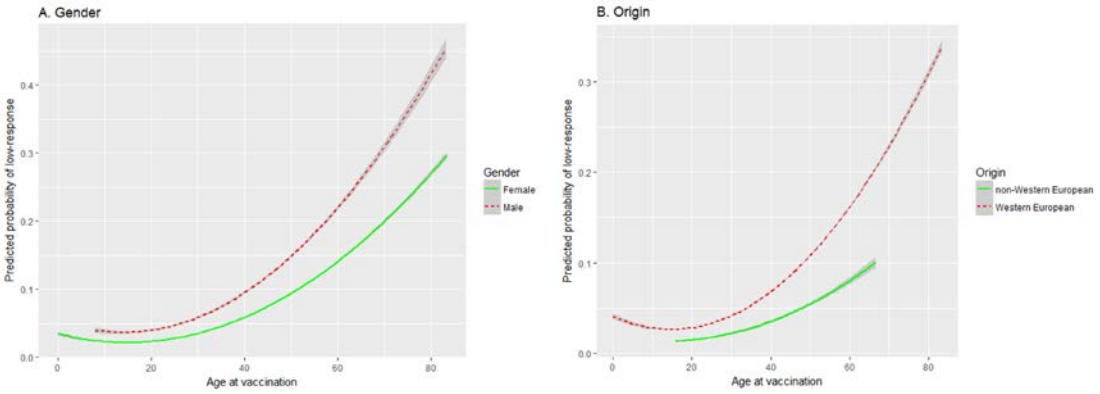


Fig. 3. Predicted low-response to hepatitis B vaccination over age at vaccination for (A) males and females, and (B) Western and non-Western European origin



Tables

Table 1 Baseline characteristics of all identified subjects (N = 11,567)		
Characteristic	**No. / mean (95% CI)**	**%**
Age at vaccination, years	27.5 (27.23 – 27.72)	
Male gender	2,733	23.6
Dutch country of birth	11,497	99.4
Western European origin	10,812	93.5
Recombinant hepatitis B vaccination	11,564	99.97
Weeks between 1st and 2nd vaccination	5.4 (5.02 – 5.86)	
Weeks between 2nd and 3rd vaccination	23.8 (23.51 – 24.02)	
Weeks between 3rd vaccination and anti-HBs level determination	6.5 (6.39 – 6.51)	

Abbreviation: CI: confidence interval; anti-HBs: antibodies against hepatitis B surface antigen.

Table 2 Comparison of characteristics between hepatitis B vaccine non-responders and responders in all identified subjects.

Characteristic	**Non-responders (n= 180)**	**Responders (n= 11,387)**	***p*-value**	**OR (95% CI)**
Age at vaccination in years, mean (95% CI)	42.3 (39.87 – 44.64)	27.2 (27.00 – 27.49)	<.001	--
Male gender, n (%)	76 (42.2)	2,657 (23.3)	<.001	2.40 (1.76 – 3.27)
Dutch country of birth, n (%)	180 (100.0)	11,317 (99.4)	.189	1.11 (0.14 – 36.45)
Western European origin, n (%)	171 (95.0)	10,641 (93.5)	.163	1.20 (0.66 – 2.44)

Recombinant hepatitis B vaccination, n (%)	180 (100.0)	11 384 (99.97)	.793	--
Weeks between 1st and 2nd vaccination, mean (95% CI)	5.7 (4.86 – 6.53)	5.4 (5.00 – 5.87)	.777	--
Weeks between 2nd and 3rd vaccination, mean (95% CI)	27.8 (24.29 – 31.25)	23.7 (23.45 – 23.96)	<.001	--
Weeks between 3rd vaccination and anti-HBs level determination, mean (95% CI)	9.2 (8.32 – 10.17)	6.4 (6.34 – 6.46)	<.001	--

Abbreviation: OR: odds ratio; CI: confidence interval; anti-HBs: antibodies against hepatitis B surface antigen.

Non-responders were defined as individuals with anti-HBs level < 10 mIU/mL, while responders had anti-HBs levels > 10 mIU/mL.

Table 3 Comparison of characteristics between hepatitis B vaccine low-responders and good-responders.

Characteristic	**Low-responders (n= 549)**	**Good-responders (n= 10,838)**	***p*-value**	**OR (95% CI)**
Age at vaccination in years, mean (95% CI)	37.99 (36.73 – 39.25)	26.70 (26.46 – 26.94)	<.001	--
Male gender, n (%)	180 (32.79)	2,477 (22.86)	<.001	1.65 (1.37 – 1.98)
Dutch country of birth, n (%)	548 (99.82)	10,769 (99.36)	.217	1.76 (0.47 – 11.92)
Western European origin, n (%)	534 (97.27)	10,107 (93.26)	<.001	2.55 (1.57 – 4.48)
Recombinant hepatitis B	549 (100.00)	10,835 (99.97)	.655	0.15 (0.02 – 6.88)

vaccination, n (%)				
Weeks between 1st and 2nd vaccination, mean (95% CI)	5.7 (4.5 – 6.8)	5.4 (5.0 – 5.9)	.782	--
Weeks between 2nd and 3rd vaccination, mean (95% CI)	23.9 (22.8 – 25.0)	23.7 (23.4 – 24.0)	.931	--
Weeks between 3rd vaccination and anti-HBs level determination, mean (95% CI)	8.2 (7.7 – 8.7)	6.3 (6.3 – 6.4)	<.001	--

Abbreviation: OR: odds ratio; CI: confidence interval; anti-HBs: antibodies against hepatitis B surface antigen.

Low-responders were defined as individuals with anti-HBs level between 10 and 99 mIU/mL, while good-responders had anti-HBs levels > 100 mIU/mL.

Table 4 Hepatitis B antibody levels by baseline characteristics of all identified subjects (N = 11,567)

Characteristic	**Anti-HBs levels (95% CI)**	***p-value***
Age at vaccination		<.001
< 40 years	912.8 (898.4 – 927.2)	
> 40 years	696.9 (671.8 – 722.0)	
Male gender		<.001
No	884.1 (869.1 – 899.1)	
Yes	806.9 (785.0 – 828.8)	
Dutch country of birth		.919
No	871.7 (811.2 – 932.2)	

Yes	865.8 (853.1 – 878.5)	
Western European origin		.054
No	907.8 (875.1 – 940.4)	
Yes	862.9 (849.6 – 876.2)	

Abbreviation: CI: confidence interval; anti-HBs: antibodies against hepatitis B surface antigen.