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Titers of antibodies to the surface antigen of hepatitis B virus after vaccination in relation to immunity-related gene variants

A prospective study among hemodialysis patients

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KEY WORDS

anti-HBs antibody, hemodialysis, interleukin, single nucleotide variant, vaccination against hepatitis B virus

ABSTRACT

INTRODUCTION Hemodialysis (HD) patients show a weaker response to hepatitis B virus (HBV) vaccination than the healthy population. Several gene variants were reported to be associated with the levels of antibodies to HBV surface antigen (anti-HBs) after HBV vaccination among healthy individuals.

OBJECTIVES The aim of the study was to determine the effect of immunity-related genes on the maximum anti-HBs antibody levels after vaccination among HD subjects.

PATIENTS AND METHODS This 6-year prospective study included HD patients who were not infected with HBV and underwent HBV vaccination. Before the study, patients were classified as responders (anti-HBs ≥10 IU/l, n = 356) or nonresponders (anti-HBs <10 IU/l, n = 48) to HBV vaccination. Patients were tested for the following gene variants: *GC* rs7041, rs1155563, rs2298849; *RXRA* rs10881578, rs10776909, rs749759; *VDR* rs1544410, rs2228570; *IFNL3* rs8099917, rs12979860; *IL12A* rs568408; *IL12B* rs3212227; *IL4R* rs1805015; *IL13* rs20541; *IL18* rs360719; and *CCL2* rs1024611. Anti-HBs titers were checked every 6 to 12 months and the individual maximum values were used in the analysis.

RESULTS There was a significant difference in peak anti-HBs levels between patients with 2 major alleles of *IL12A* rs568408 (median, 180 IU/l; range, 0–4.105 IU/l) and those carrying 1 or 2 minor alleles (median, 451 IU/l; range, 0–5.342 IU/l; $P = 0.004$). In a multivariate analysis, a positive correlate of the maximum anti-HBs antibody titers was dialysis duration, while the negative ones included the GG genotype of *IL12A* rs568408, age, and time elapsed from dialysis onset to peak anti-HBs antibody titers.

CONCLUSIONS In HD patients, peak anti-HBs levels following vaccination are independently associated with the *IL12A* rs568408 variant.

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INTRODUCTION The prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) among hemodialysis (HD) patients remains higher than in the healthy population.[1,2] Despite advanced hepatitis B vaccination schedule,[3] HD patients more often do not develop protective antibody levels, and even if they do, the levels decrease more quickly than among the healthy population.[4] Furthermore, the peak titers of protective antibodies may be different in individual HD patients. It is crucial to identify factors associated with attenuated response to vaccination to identify people with an increased risk of not responding or weakly responding to immunization, especially given the fact that these patients present worse survival during HD therapy.[5]

In previous publications, the minor allele of interleukin (*IL*)1β (+3953) was found to be associated with significantly higher titers of antibodies to the surface antigen of hepatitis B virus (anti-HBs) after HBV vaccination.[6] In an analysis of a conjoint influence of 3 single nucleotide variants (SNVs) in the promoter of *IL10* at the positions –1082, –819, and –592, patients with the

ACC haplotype were found to have significantly increased anti-HBs antibody titers after vaccination.[7] A haplotype analysis examining 688 variants in 117 genes identified haplotypes of 5 genes that significantly affected the peak anti-HBs antibody titers, 2 of which were genes of ILs (*IL19*: rs12409415-rs256225-rs2243158 and *IL1R1*: rs2287047-rs997049-rs3917299).[8]

Out of the 10 examined SNVs in the genes of cytokines, only the variants in *IL12A* and *IL12B* were found to significantly affect the postvaccination levels of anti-HBs antibodies in the Chinese population.[9] These results do not fully corroborate those obtained by Lin et al[10] in the Taiwanese population, where rs2243250 in *IL4* was found to affect the antibody titers, with the CC carriers having significantly lower ones. A weaker association of the rs1805010 SNV in *IL4R* with the anti-HBs antibody titers was also shown.[10] Also other SNVs than those of IL genes were reported to affect postvaccination anti-HBs antibody titers, including SNVs in the genes of G protein β3,[11] HLA-DQB1,[11] and HLA-DPB1.[12]

In this study, we investigated the association of SNVs in genes related to immune response with the peak levels of anti-HBs antibodies after hepatitis B vaccination or booster vaccine doses in patients treated with intermittent HD. Dialyzed patients tend to receive multiple booster doses during their long-term dialysis treatment, which improves the response rate to HBV vaccination in nonresponders to the primary vaccination,[13,14] but it is not clear whether they might affect anti-HBs antibody titers in HD patients, and, if so, for how long during HD treatment. Therefore, the strength of the immune response to HBV vaccination could be assessed. Also, the number of patients with anti-HBs antibody titers equal to or higher than 1000 IU/l was counted, due to the fact that such postvaccination anti-HBs antibody levels were reported to prolong the durability of protective antibody concentrations among immunocompromised patients when compared with lower postvaccination anti-HBs antibody titers,[15] and were considered to be a marker of excellent response.[16] The tested SNVs were associated with the vitamin D signaling pathway (group-specific component protein [*GC*] rs7041, rs1155563, rs2298849; retinoid X receptor α [*RXRA*] rs10881578, rs10776909, rs749759; vitamin D receptor [*VDR*] rs1544410, rs2228570), Th1 cytokines (interferon λ3 [*IFNL3*] rs8099917, rs12979860; *IL12A* rs568408; *IL12B* rs3212227), Th2 cytokines (*IL4R* rs1805015; *IL13* rs20541; *IL18* rs360719; and C-C motif chemokine ligand 2 [*CCL2*] rs1024611). Thus far, there have been no studies determining their influence on the peak anti-HBs antibody titers after vaccination in HD patients.

PATIENTS AND METHODS

Patient enrollment

This observational prospective study started in January 2009 and included HD patients inhabiting the region of Wielkopolska in Poland, who had not undergone renal transplantation before the study (n = 532). It was the same cohort that was previously described in terms of factors that affect survival among HD subjects.[5,17] A history of HBV vaccinations and potential hepatic diseases was taken at the start of HD treatment. Additionally, all patients were tested for the HBV infection status by checking their HBV surface antigen (HBsAg) and total antibodies to HBV core antigen (anti-HBc) as a routine approach in the HD population. Those who were noninfected with HBV were vaccinated against HBV in accordance with the full vaccination program for HD patients.[18] Patients who were vaccinated before the start of HD treatment, but showed anti-HBs antibody titers below 10 IU/l, were given 1 or more booster doses. In case of insufficient response to the primary vaccination (anti-HBs <10 IU/l), patients were given at least 3 further vaccine doses (see the "Hepatitis B virus vaccination" section for details). Those with anti-HBs antibody levels equal to or exceeding 10 IU/l were classified as responders to HBV vaccination, whereas those with anti-HBs antibody levels lower than 10 IU/l were considered as nonresponders. If the patients attained anti-HBs antibody titers equal to or exceeding 10 IU/l at least once in their lifetime, they were qualified as able to respond to HBV vaccination, even if later the titers decreased below 10 IU/l. Therefore, when the study began, all patients had already been vaccinated and their responsiveness to HBV vaccination was known. In all cases, the vaccine used was the one containing recombinant HBsAg derived from yeast (Engerix B, GlaxoSmithKline Biologicals, Rixensart, Belgium; Hepavax-Gene TF, BIOMED SA, Poland; Euvax B, LG Life Sciences, Warsaw, Poland).

All patients had 3 dialysis sessions a week. Online hemodiafiltration, low-flux HD, or high-flux HD were applied. Standard medicines and diet for HD patients were prescribed.

Hepatitis B virus vaccination

Primary vaccination program was completed according to the schedules as follows: if patients were vaccinated with Engerix B (n = 376; 93%), they were immunized with 4 doses of 40 μg each: at baseline and after 1, 2, and 6 months. Patients vaccinated with Hepavax-Gene TF (n = 16; 4%) or Euvax B (n = 12; 3%) were administered 3 doses of 40 μg each: at baseline and after 1 and 6 months. If after that program the patients did not achieve protective anti-HBs antibody titers (≥10 IU/l), they were administered further vaccine doses (40 μg) until the titers reached 10 IU/L or higher, or until the patients were administered no fewer than 7 vaccine doses in total.

If anti-HBs antibody levels became lower than 10 IU/l, the responders were given a booster vaccination (40 μg). In some cases, standard vaccination doses (20 μg) were administered to those whose anti-HBs antibody levels started falling and getting close to 10 IU/l. After the administration of each vaccine dose, anti-HBs antibody levels were measured within 4 to 6 weeks.

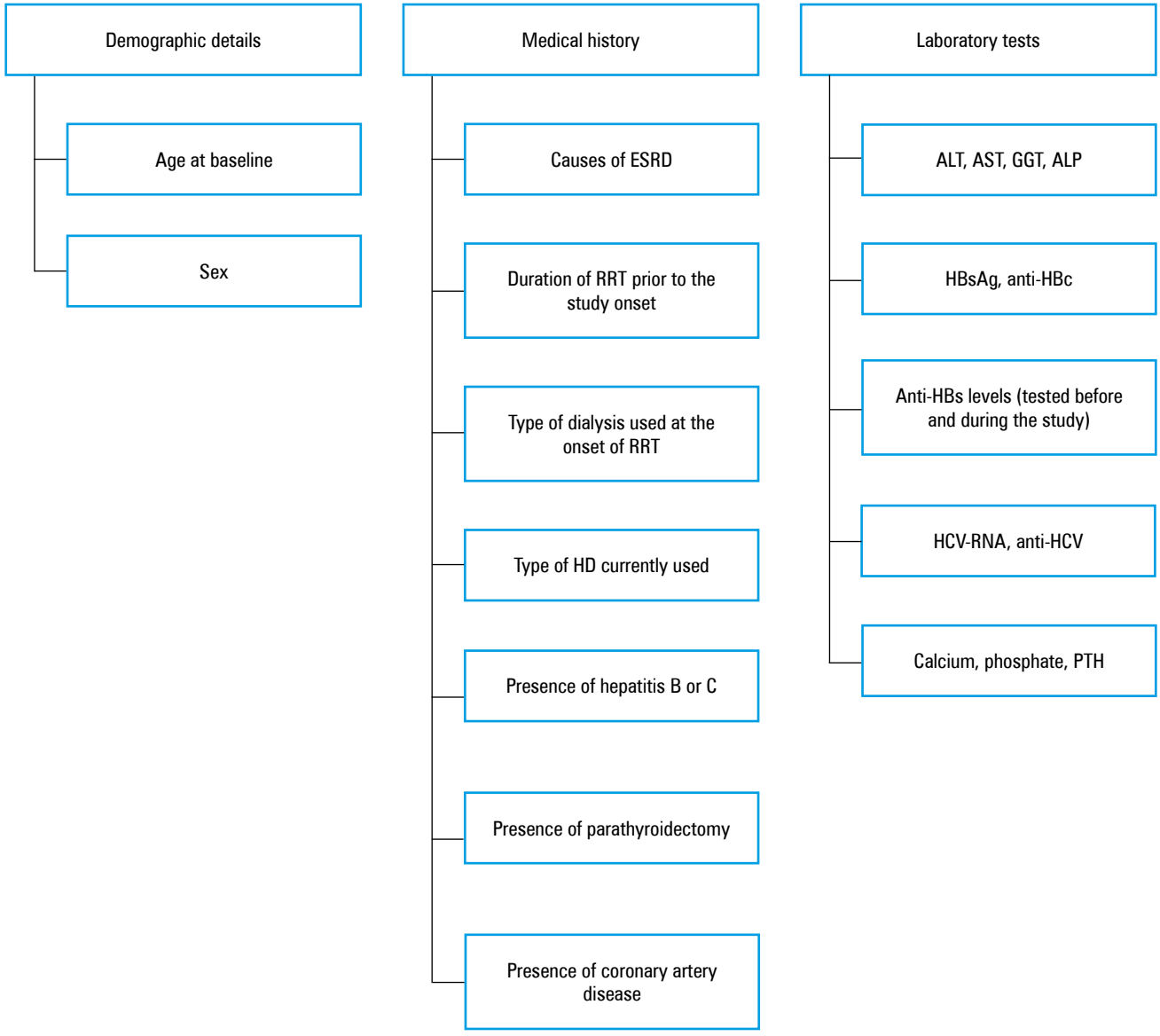

FIGURE 1 Baseline demographic, clinical, and laboratory data of hemodialyzed patients
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-HBc, antibodies to hepatitis B virus core antigen; anti-HBs, antibodies to hepatitis B surface antigen; anti-HCV, antibodies to hepatitis C virus; AST, aspartate aminotransferase; ESRD, end-stage renal disease; GGT, γ-glutamyl transferase; HBsAg, hepatitis B virus surface antigen; HCV-RNA, hepatitis C virus ribonucleic acid; HD, hemodialysis; PTH, parathyroid hormone; RRT, renal replacement therapy

As a standard, anti-HBs antibody titers were measured once or twice a year (prior to the start of the study and during the entire study). The highest anti-HBs antibody levels attained by a patient before the study onset or within its duration were registered as peak (maximum) anti-HBs antibody levels. Also, the number of patients whose anti-HBs antibody titers were equal to or exceeded 1000 IU/l was noted.

Study details The study started on January 30, 2009. The data of patients were collected at baseline (FIGURE 1). The peak anti-HBs antibody titers were assessed during a 6-year follow-up (until January 30, 2015). Additionally, data on anti-HBs antibody titers of patients before the study onset were collected. During the study, patients were regularly monitored for markers of HBV and HCV infection.

Laboratory methods Anti-HBs antibody titers were determined with a microparticle enzyme immunoassay (ABBOTT, Wiesbaden, Germany) or a chemiluminescent microparticle immunoassay (ABBOTT, Sigo, Ireland). Standard laboratory methods were used for other tested parameters.

Genotyping Patients underwent genetic testing for the SNVs in the following genes: *GC* (rs7041, rs1155563, rs2298849), *RXRA* (rs10881578, rs10776909, rs749759), *VDR* (rs1544410, rs2228570), *IFNL3* (rs8099917, rs12979860), *IL12A* (rs568408), *IL12B* (rs3212227), *IL4R* (rs1805015), *IL13* (rs20541), *IL18* (rs360719), and *CCL2* (rs1024611). The characteristics of the

TABLE 1 Baseline demographic, clinical, and laboratory characteristics of responders to HBV vaccination (n = 356)

Parameter	Value
Demographic data	
Male sex, n (%)	201 (56.4)
Age at baseline, y, median (range)	61.2 (14.6–89.3)
RRT vintage at baseline, y	2.3 (0.0–15.2)
Cause of ESRD, n (%)	
Diabetic nephropathy	95 (26.7)
Chronic glomerulonephritis	66 (18.5)
Hypertensive nephropathy	63 (17.7)
Chronic tubulointerstitial nephritis	50 (14.0)
Clinical data, n (%)	
Coronary artery disease	141 (40.2)
History of HCV infection (anti-HCV positivity)	30 (8.4)
HCV-RNA positivity	17 (4.8)
Type of RRT, n (%)	
LF-HD	183 (51.4)
HF-HD	146 (41.0)
HDF	27 (7.6)
Laboratory data, median (range)	
ALT, IU/l	13.0 (3.0–131.0)
AST, IU/l	14.0 (3.0–177.0)
GGT, IU/l	26.0 (1.0–682.0)
PTH, pg/ml	414 (19.5–3757)

Conversion factors to SI units are as follows: for alanine aminotransferase, 1 U/l = 0.0167 μkat/l; for aspartate aminotransferase, 1 U/l = 0.0167 μkat/l; for γ-glutamyltransferase, 1 U/l = 0.0167 μkat/l; for parathyroid hormone, 1 pg/ml = 1 ng/l.

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HDF, hemodiafiltration; HF-HD, high-flux hemodialysis; LF-HD, low-flux hemodialysis; others, see FIGURE 1

variants are summarized in Supplementary material online, *Table S1*.

The genotyping of *IL12B*, *IL18*, *VDR* rs2228570, *VDR* rs1544410, *GC* rs7041, *RXRA* rs749759, and *CCL2* SNVs was conducted using a polymerase chain reaction analysis, whereas genotyping of the remaining SNVs was achieved using a high-resolution melting curve analysis. Details concerning genotyping can be found in our previous publications.[5,17,19,20]

Statistical analysis The continuous variables that did not follow normal distribution (according to the results of the Shapiro–Wilk test) were presented as median and range, whereas categorical variables—as numbers and percentages. The Mann–Whitney test, χ^2 test with Yates correction, Kruskal–Wallis test by ranks, and *t* test were applied to assess significant differences in the studied parameters.

All genotype distributions were checked for concordance with the Hardy–Weinberg equilibrium (HWE) using the χ^2 test ($P > 0.01$ with df = 1 for equilibrium). An analysis of associations between the tested SNVs and the maximum anti-HBs antibody titers was performed using 3 models of inheritance (dominant, recessive, and additive). Stepwise regression with forward selection was conducted to verify the effect of clinically relevant variables together with the SNVs that reached statistical significance in single analyses on the peak anti-HBs antibody titers. The results include a raw regression coefficient (B) with standard error that shows the contribution of the independent variable to the maximum anti-HBs antibody titers. The B coefficient reflects a change in anti-HBs antibody titers that would result from a unitary change of an independent variable.

A *P* value of less than 0.05 was considered significant. For multiple comparisons, the *P* value was adjusted for the Bonferroni correction ($P < 0.017$ was considered significant for 3 groups). STATISTICA version 12 (Stat Soft, Inc., Tulsa, Oklahoma, United States) and Graph-Pad InStat 3.10, 32 bit for Windows (GraphPad Software, Inc., San Diego, California, United States) were used for statistical analysis.

Ethical approval and written consent All procedures involving human participants were performed in accordance with the ethical standards of the Institutional Review Board of the Poznan University of Medical Sciences and with the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all study participants.

RESULTS

Response to hepatitis B virus vaccination Among all study participants (n = 532), 404 patients were not infected with HBV. There were 356 patients (88.1%) who developed sufficient anti-HBs antibody levels (≥10 IU/l) after vaccination. The main characteristics of these patients are presented in TABLE 1.

Among the 356 vaccine responders, 4 patients (1%) developed the maximum anti-HBs antibody titers before the onset of dialysis treatment; 211 patients (59%), during the first 5 years of dialysis treatment; 115 patients (32%), between >5 and 10 years of dialysis duration; and the remaining 26 patients (7%), after >10 years since the study onset (FIGURE 2). The median peak anti-HBs antibody titers attained at different time points with regards to the onset of dialysis did not differ between the 4 groups of patients ($P = 0.22$; FIGURE 3).

All HD patients developed peak anti-HBs antibody levels after a median period of 4.22 years (–1.31 to 17.33 years from dialysis onset). HBV-vaccine responders achieved their highest levels of anti-HBs antibodies after a median period of 1.59 years (4.07 to 6.00 years from dialysis onset). The median peak anti-HBs antibody titer was 334 IU/l (range, 10–5342 IU/l). Among the responders, 84 patients (23.6%) developed anti-HBs antibody titers equal to or exceeding 1000 IU/l. Patients who survived the 6-year follow-up developed their maximum anti-HBs antibody titers later than those who died (median, 5.06; range, 0–17.01 vs median, 3.49; range, –1.31 to 17.34 years from dialysis onset, $P = 0.004$).

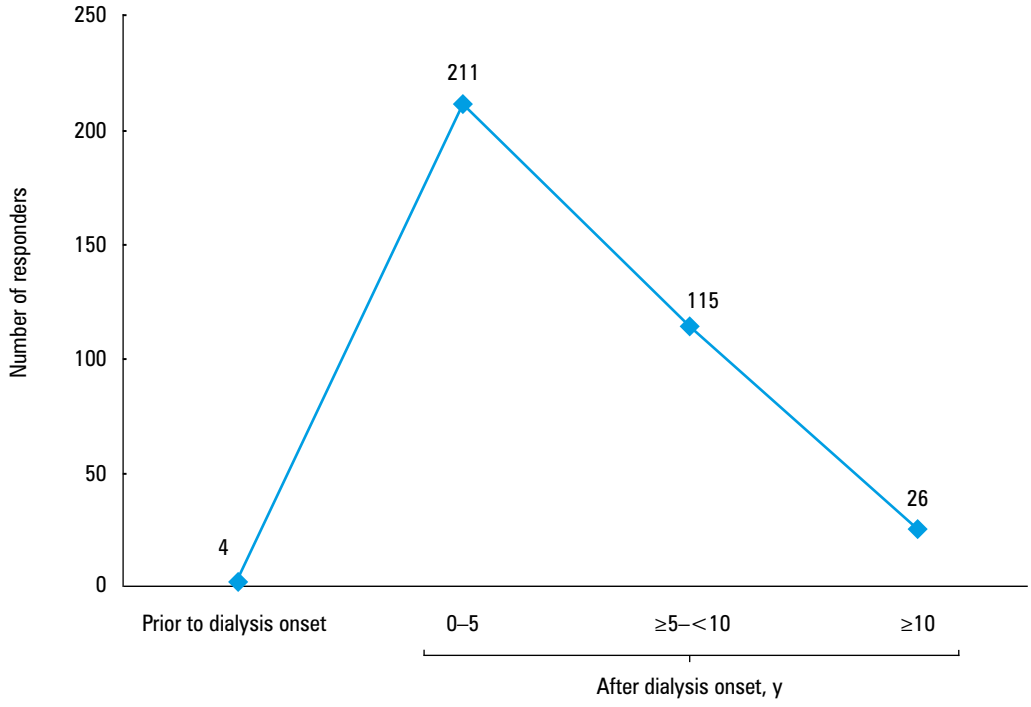

FIGURE 2 Number of responders (n = 356) who developed maximum levels of antibodies to hepatitis B surface antigen depending on time elapsed from dialysis onset

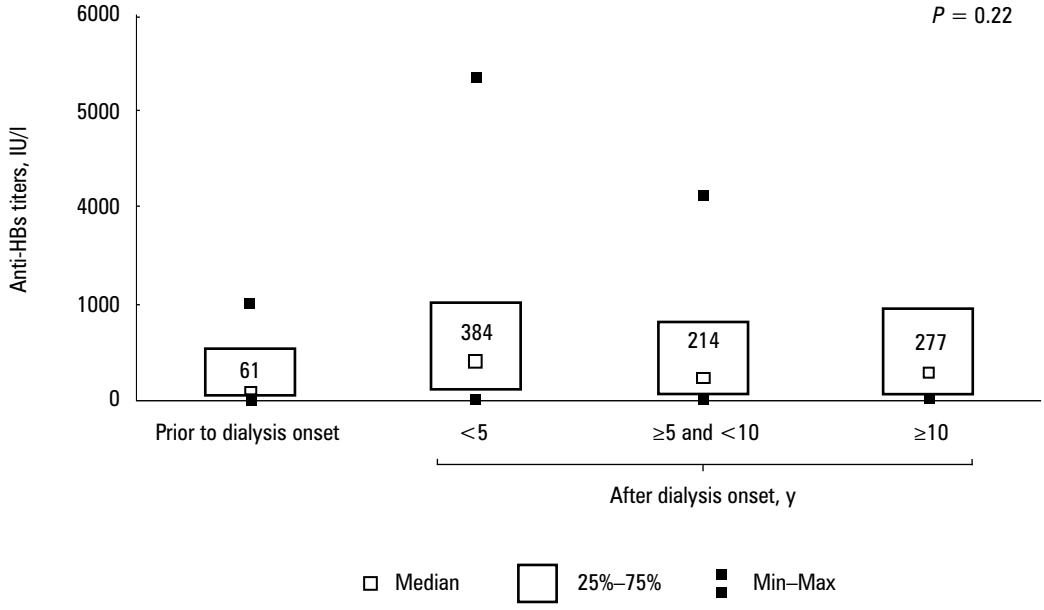

FIGURE 3 Maximum levels of antibodies to hepatitis B surface antigen (anti-HBs) depending on time elapsed from dialysis onset among responders to hepatitis B virus vaccination (n = 356); a *P* value calculated using the Kruskal–Wallis test. Abbreviations: see **FIGURE 1**

Concordance with the Hardy–Weinberg equilibrium The distribution of genotypes within the group of patients noninfected with HBV (n = 404) was concordant with the HWE for all the SNVs apart from *GC* rs1155563 and *GC* rs2298849 (Supplementary material online, *Table S2*). For the whole study group (patients both infected and noninfected with HBV, n = 532), the distribution only of *GC* rs1155563 was not in agreement with the HWE. A comparison of the genotype distributions of all vaccinated patients (n = 404) with those of healthy controls described in our previous paper[20] revealed a difference only for the *GC* rs1155563 SNV: $P_{trend} = 0.001$, $P_{genotype} = 0.01$, $P = 0.013$ for the additive model of inheritance (Supplementary material online, *Table S3*).

Anti-HBs antibody titers in relation to the tested single nucleotide variants The maximum anti-HBs antibody titers analyzed in relation to the tested SNVs in all patients vaccinated against HBV were significantly different only for the *IL12A* rs568408 SNV (Supplementary material online, *Table S4*). They were lower in carriers of 2 major alleles of

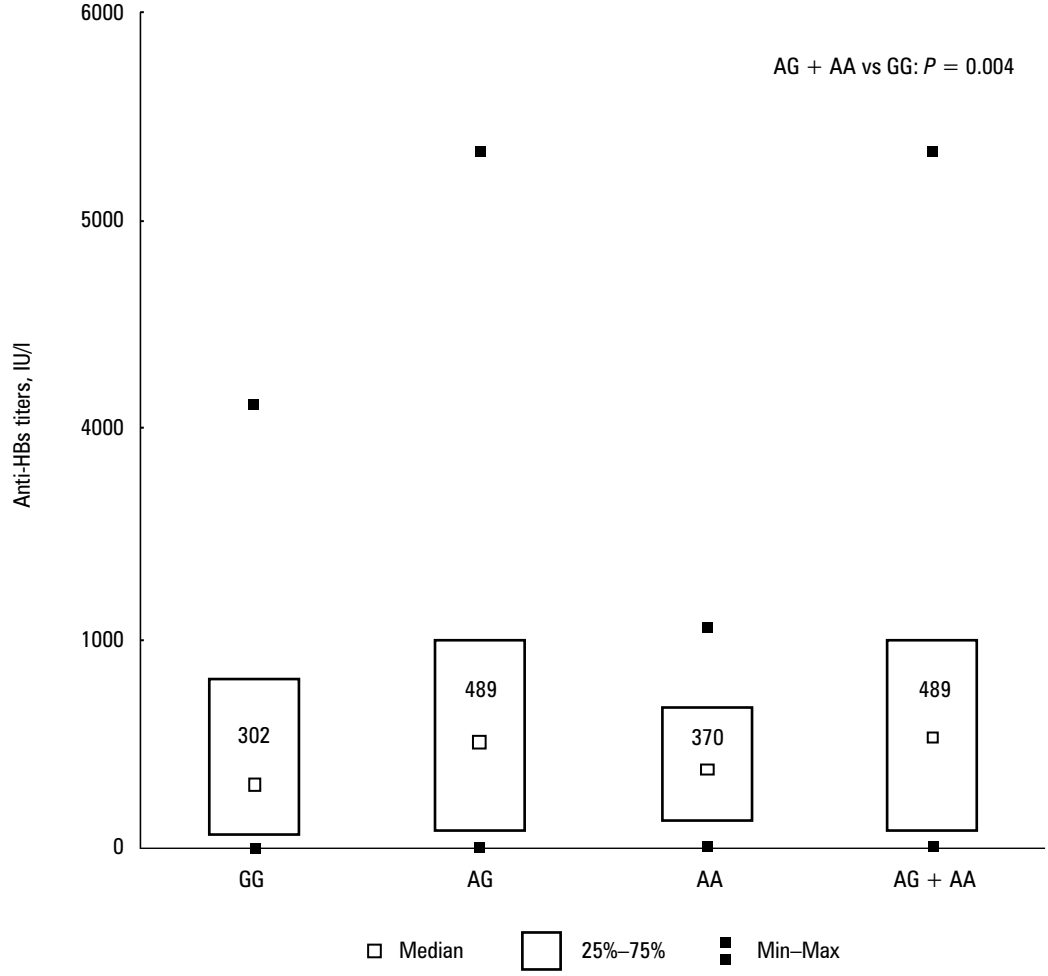

FIGURE 4 Maximum levels of antibodies to hepatitis B surface antigen (anti-HBs) after vaccination according to genotypes of the *IL12A* rs568408 variant among responders to hepatitis B virus vaccination (n = 356); a *P* value calculated using the Mann–Whitney test. Abbreviations: see **FIGURE 1**

IL12A rs568408 than in those of 1 or 2 minor alleles (a dominant model of inheritance; **FIGURE 4**).

In the analysis of the number of patients with anti-HBs antibody levels equal to or greater than 1000 IU/l, a trend for difference was also observed only for the *IL12A* rs568408 variant (Supplementary material online, *Table S5*). Carriers of at least 1 minor allele were more likely to develop anti-HBs antibody levels equal to or exceeding 1000 IU/l (28% vs 17%; odds ratio, 1.823; 95% CI, 1.068–3.113; *P* = 0.04; not significant after the Bonferroni correction).

The analysis of dialysis vintage at the time of reaching peak anti-HBs antibody levels among carriers of different genotypes of each SNV showed that carriers of at least 1 minor allele of *IL12B* rs3212227 developed maximum anti-HBs antibody titers later (after a median period of 5.16 years and ranging from 1.15 years before to 17.34 after dialysis onset) than major homozygotes, who developed peak anti-HBs antibody titers after a median period of 4.02 years, ranging from 1.31 years before to 17.01 years after the dialysis onset (*P* = 0.007; Supplementary material online, *Table S6*).

No other significant associations were observed between the maximum anti-HBs antibody titers and the tested SNVs in the immunity-related genes (Supplementary material online, *Tables S4* and *S5*).

Anti-HBs antibody titers in relation to other clinical variables In the stepwise regression analysis with forward selection, the peak anti-HBs antibody level was analyzed as a dependent variable and the GG genotype of *IL12A* rs568408, age, dialysis vintage, sex, type of dialysis treatment, diabetic nephropathy, parathyroid hormone levels, and time span to peak anti-HBs antibody levels were considered as explanatory variables for the maximum anti-HBs antibody titers. The analysis revealed that a positive correlate of the maximum anti-HBs antibody titers was dialysis vintage, while the negative ones included the GG genotype of *IL12A* rs568408, age, and time elapsed from dialysis onset to peak anti-HBs antibody titers (**TABLE 2**). All the remaining factors were nonsignificant.

DISCUSSION According to recent studies,[21,22] approximately 80% of HD patients respond to HBV vaccination. The response rate of 88.1% obtained in our cohort was slightly higher, but we should allow for the fact that our patients were

TABLE 2 Explanatory variables for the peak levels of antibodies to hepatitis B surface antigen among demographic, genetic, and clinical parameters

Parameter	Peak anti-HBs antibody titers	
	B[a] ± SE	*P* value
Age, per 10 years	–7.5820 (2.2388)	0.0008
GG genotype of *IL12A* rs568408	–162.1430 (74.1138)	0.03
Time span to peak anti-HBs titers, y	–37.4700 (15.8191)	0.02
Dialysis vintage, per 1 year	33.7130 (16.5887)	0.04

a B coefficient reflects the change in peak anti-HBs antibody titers for each unitary change in the independent variable.

A *P* value of less than 0.05 was considered significant. The statistical method: stepwise regression with forward selection (multiple $r = 0.274$, $P = 0.0004$)

Abbreviations: SE, standard error; others, see **FIGURE 1**

categorized as nonresponders if they did not develop protective anti-HBs titers after receiving at least 7 vaccine doses, which significantly improves responsiveness to vaccination. Among healthy individuals, 3 booster doses were reported to induce 100% response in nonresponders to the primary vaccination schedule.[13] In immunocompromised patients infected with human immunodeficiency virus, booster doses administered to nonresponders increased the rate of response from 60% after primary vaccination to 89.4% after up to 3 booster doses,[14] which is similar to the response rate obtained among our participants.

Generally, older patients were rarely vaccinated before the onset of dialysis treatment.[23] As the subject of this study concerns maximum anti-HBs titers, it is important to note that only 1% of the patients obtained their peak anti-HBs levels prior to dialysis onset. Nowadays, after the introduction of compulsory HBV vaccination of children, the rate of HBV-vaccinated pediatric patients before dialysis onset reaches 68%.[24]

In our cohort, HD patients developed peak anti-HBs levels after a median period of 4.22 years after the dialysis onset, while the primary vaccination schedule was usually carried out at the beginning of dialysis treatment. Achieving the maximum anti-HBs concentrations after such a period of time may be caused by the fact that our patients were given booster doses in the case of losing or approaching protective anti-HBs concentrations. Even one booster dose administered to patients with chronic kidney disease with titers lower than 100 IU/l after the primary vaccination was found to significantly increase anti-HBs concentrations among 57% of the patients.[25] Besides repeated active immunizations, an increase in anti-HBs levels could result from contact with HBV. Bulkow et al[26] reported an increase in anti-HBs levels during a 10-year observational study among 8% of subjects initially vaccinated against HBV, which could be explained only by exposure to HBV. However, such a possibility was limited in our study by the fact that we measured anti-HBs levels shortly after the administration of a booster dose.

The percentage of healthy individuals with anti-HBs titers equal to or exceeding 1000 IU/l, referred to as hyperresponders[27] or high-responders,[9] was reported as 9.2% for Hepavax Gene, 33.8% for Engerix, and 37.9% for Euvax B.[27] These vaccines contain 20-μg doses of anti-HBs, but were produced by different yeast strains.[27] All these results, except for those for Hepavax Gene,[27] are higher than the ones obtained in our study (23.6%). Despite this, these values are quite high as for individuals with altered immunocompetence. On the other hand, HD patients received additional booster doses if necessary, and in our study, only peak anti-HBs concentrations were registered, whereas in the study by Hernandez-Bernal et al,[27] the anti-HBs titers were monitored after a year from completing an accelerated vaccination schedule (20-μg doses at 0, 1, and 2 months).

To our knowledge, there have been no studies that investigated peak anti-HBs titers and their association with the immunity-related genes among HD patients. As most studies that investigated the influence of selected SNVs on the peak anti-HBs levels after vaccination were conducted among healthy individuals, our study is one of the few that assessed them among individuals with compromised immune response. Moreover, we monitored anti-HBs titers for a prolonged period of time after the first vaccination or next booster doses, unlike most studies, which focused only on response shortly after the standard vaccination course. Such monitoring seems to be particularly significant because the responder status to HBV vaccination is an independent risk factor for death among HD patients.[5]

In our previous study, the *IL12A* rs568408 SNV was reported to be associated with the responder status to HBV vaccination (anti-HBs titers <10 IU/l vs ≥10 IU/l) among HD patients (not significant after the Bonferroni correction).[19] Our current results indicated an association between peak anti-HBs levels obtained during dialysis and the GG *IL12A* rs568408 SNV in HBV-vaccinated patients on HD. This genotype was also found to be an independent negative predictive factor of the peak anti-HBs concentration. Moreover, there was a borderline association for the number of hyperresponders (anti-HBs titers ≥1000 IU/l) and *IL12A* rs568408: there were fewer such subjects among the carriers of 2 major alleles of this SNV than among the carriers of at least 1 minor allele. The *IL12B* rs3212227 SNV did not affect peak anti-HBs levels after vaccination. Nonetheless, major homozygotes of this SNV developed the maximum anti-HBs titer significantly faster than individuals with at least 1 minor allele.

IL-12 is one of the Th1-pathway cytokines, thus promoting interferon-γ production and enhancing the cytotoxicity of lymphocytes. The active isoform of IL-12 is the p70 heterodimer that consists of IL-12 p35 and IL-12 p40 (encoded by *IL12A* and *IL12B*, respectively).[28] The rs568408 SNV is within the 3' untranslated region of *IL12A*. The

effect of this SNV on the circulating IL-12 levels has not yet been studied. It was only shown that the *IL12B* rs3212227 SNV affected the circulating levels of IL-12 p40.[29,30]

Among other immune response abnormalities in uremic patients, the secretion of IL-12 was reported to be disturbed. In vitro studies showed that dendritic cells of HD patients secrete more IL-12 p70, the active form of IL-12 (after incubation both in uremic serum and serum of healthy individuals), and that the functioning of these cells is significantly impaired.[31,32] Furthermore, HD patients were reported to have significantly more Th1-polarized cells than healthy controls with an increased IL-12 secretion.[33,34] As Th2 immune response is associated with antibody production, this Th1 skewing might be one of the reasons behind the attenuated response to vaccinations among uremic patients, achieved through an increased IL-12 production, which may be affected by the *IL12A* rs568408 variant. This might explain the influence of this SNV on peak anti-HBs titers among HD patients.

SNVs other than *IL12A* rs568408 and *IL12B* rs3212227 were also reported as affecting responsiveness to HBV vaccination.[9] The particular genotypes of *IL12A* rs2243115 and *IL12B* rs17860508 were jointly associated with lower anti-HBs levels after vaccination of healthy individuals in a Chinese Han population. Therefore, further studies might allow an identification of the genotype sets of different variants that, when combined together, affect anti-HBs titers after vaccination.

Time elapsed from dialysis onset to achieving the maximum anti-HBs concentrations was an independent negative predictive factor of peak anti-HBs titers, which suggests that if a patient developed the peak titers later, they tended to be lower. The patient's age was another independent negative predictive factor: the peak anti-HBs levels decreased with increasing age. This is concordant with the results of a meta-analysis that linked older age with a lower rate of response to HBV vaccination, regardless of booster doses.[35] Surprisingly, dialysis vintage was a positive predictive factor. It has been shown that patients at earlier stages of chronic kidney disease respond significantly better to HBV vaccination.[36] However, in a different study, dialysis vintage was not found to be a significant independent factor in a multivariate analysis that affected response to the vaccine.[37] In our study, patients were administered multiple booster doses, so some of them could develop maximum anti-HBs levels after a longer period of dialysis treatment as an effect of these subsequent doses of HBV vaccination.

Interestingly, patients who survived the 6-year follow-up period developed the maximum anti-HBs titers significantly later (counting from the dialysis onset) than those who died. It might be caused by a variety of reasons, such as longer dialysis duration and therefore a bigger number of booster doses received, but also by preserving the ability to produce high antibody concentrations in response to antigens for longer time among those who survived in contrast to those who died. Response to HBV vaccination was found to be a positive factor of survival among HD patients.[5] Moreover, carriers of at least one A allele of the *IL12A* rs568408 SNV were reported to have significantly lower all-cause mortality rates when compared with GG carriers,[38] and, according to our results, they had significantly higher peak anti-HBs titers. Also, there were fewer responders among GG carriers to HBV vaccination,[38] and in our study, the GG genotype was a negative correlate of the maximum anti-HBs concentrations. Therefore, the association between the genotype of *IL12A* rs568408 and postvaccination anti-HBs concentrations might have clinical implications for the survival of HD patients.

Limitations of the study There was no concordance with HWE for 2 *GC* variants (rs1155563 and rs2298849). It might be explained by the fact that we investigated a highly selected group of individuals and not the general population. This hypothesis is partially confirmed by the fact that when the whole group of dialysis patients was analyzed (n = 532), only the *GC* rs1155563 SNV distribution was not concordant with the HWE. So far, the minor allele of this SNV was linked with lower circulating vitamin D levels.[39,40] In our study, there were significantly more minor homozygotes of *GC* rs1155563 in dialysis patients compared with controls (Supplementary material online, *Table S3*). It is not surprising because vitamin D deficiency is a common feature in HD patients,[41] and it was also observed in the studied subjects.[5]

Another limitation of the study is that patients were vaccinated using 3 different vaccines. However, an overwhelming majority of the patients were vaccinated using Engerix B, and they were administered booster doses if they failed to respond to the primary schedule. Therefore, the potential effect of using different vaccines was minimized. Owing to the small sample size of Euvax and Hepavax Gene, it was impossible to verify whether there were any differences in the peak anti-HBs titers between the 3 groups.

Among the vaccinated participants, 9% of the patients underwent hepatitis C infection, which might have affected the response to HBV vaccination. However, a meta-analysis by Fabrizi et al[42] showed no association between anti-HCV positivity and response to HBV vaccination among HD patients; therefore, we decided to include HCV-positive patients in our analysis.

Supplementary material online Supplementary material is available with the online version of the article at www.pamw.pl.

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