Antibody Level after Hepatitis-B Vaccination in Hemodialysis Patients: Impact of Dialysis Adequacy, Chronic Inflammation, Local Endemicity and Nutritional Status

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We prospectively studied the evolution of HBsAg antibody (HBsAb) after primary vaccination (four doses; Engerix B, 40 µg i.m at 0, one, two and six months) in 29 patients who were seronegative (HBsAb <10 IU/L), had not been previously vaccinated and were on hemodialysis. Their mean age was 45.58 ± 10.98 years, and the hemodialysis duration ranged from 1–21 years. In addition, we assessed dialysis adequacy for all cases on four different occasions beside the estimation of predialysis serum albumin, serum ferritin, C-reactive protein (CRP), transferrin saturation ratio (TSAT), body mass index (BMI) and subjective global assessment (SGA). We measured anti-HBs titer eight weeks after the fourth dose. Our results showed that two patients (6.90%) were nonresponders (HBsAb <10 IU/L) after the completion of vaccination. One patient (3.45%) was a weak responder (10–100 IU/L). Strikingly, 26 patients (89.65%) showed good antibody response (>100 IU/L). HBsAb titers showed no significant correlation with age, duration of HD therapy, serum albumin, CRP, TSAT level, BMI or SGA scores ($p>0.05$). Responders to primary vaccination had significantly higher levels of urea reduction ratio (%) and Kt/V compared to nonresponders (63.61 ± 6.97% and 1.25 ± 0.15 vs. 52.0 ± 2.10% and 0.92 ± 0.13, respectively, $P<0.05$). In conclusion, this was a preliminary study showing a very high response to hepatitis-B vaccination among hemodialysis patients that neither correlated with age, systemic inflammation nor nutritional status. Efficient hemodialysis was associated with good response to hepatitis-B vaccine.

Key words: hepatitis B ■ vaccination ■ hemodialysis ■ nutritional state ■ chronic inflammation

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INTRODUCTION

Patients on hemodialysis therapy are at a relatively high risk of exposure to hepatitis-B virus (HBV) infection.[1] HBV is present in extraordinary high titers in blood and other bodily fluids of infected patients.[2] Dialysis staff can transfer the virus to patients from contaminated surfaces via their hands or through use of contaminated equipment and supplies.[2] HBV infection is a major public health problem in the Middle East; the majority of its countries have an intermediate or high endemicity of HBV infection, including Egypt.[3]

Hepatitis-B vaccine has been recommended for all susceptible hemodialysis patients since it became available in 1982, but the seroconversion rates are much lower in the patient population with end-stage renal disease (ESRD).[4] Seroconversion (anti-HBs >10 IU/l) was found in 73–76.7% of hemodialysis patients three months after vaccine administration, but an adequate response (anti-HBs >100 IU/l) was observed only in 53.5% in one series.[5] Others have also shown lower response rates to HBV vaccine that ranged from 47–58% one month after the fourth injection.[6,7]

Beside lower response rates, hepatitis-B vaccine immunogenicity is often transient, and booster doses are necessary to maintain protection against HBV.[5] Forty-one percent of responders had no detectable anti-HBs levels in the serum after three years of follow-up in one study.[5] Reasons for poor response include malnutrition, uremia, older age and the immunocompromised state of patients with chronic kidney disease.[8]

Factors that have been associated with good response to HBV vaccination include young age (<40 years),[9] good nutritional status[10] and adequacy of dialysis.[11] On the other hand, duration of dialysis,[4] hemoglobin and parathyroid hormone levels and hepatitis-C virus (HCV) infection did not significantly influence antibody responses to hepatitis-B immunization.[8]

The aim of this study was to assess the influence of nutritional status, systemic inflammation, dialysis adequacy and duration on the development of hepatitis-B antibodies in a cohort of regular hemodialysis patients.

PATIENTS AND METHODS

The study was conducted in Kasr Al-Eini Nephrolo-

gy and Dialysis Centre, Cairo University Hospital, over a period of one year (December 2003 to November 2004). We prospectively studied the evolution of HBsAg antibody (anti-HBs) after primary vaccination (four doses; Engerix B, 40 μg administered by deltoid intramuscular injection at 0, one, two and six months) in 29 patients who were seronegative (anti-HBs) <10 IU/l), had not been previously vaccinated and were on hemodialysis. All the subjects were negative for all serological markers of HBV infection, including HBsAg and anti-HBc antibodies.

The study included 19 males and 10 females. Their mean age was 45.58 ± 10.98 years (range 23–61 years) (Table 1). The etiology of end-stage renal failure was angionephrosclerosis in six cases (20.7%), interstitial nephritis in five cases (17.2%) obstructive uropathy in five cases (17.2%) and analgesic nephropathy in three cases (10.4%). The cause was unknown in 10 cases (34.5%). All the study patients were anti-HCV antibody positive.

The mean duration of hemodialysis therapy was 6.68 ± 4.93 years (range 1–21 years). Bicarbonate dialysis was performed for all patients for 3–5 hours thrice weekly. The blood flow rate ranged from 300–500 ml/min, the dialysate flow rate was 500 ml/min, and 1.3 m^2 cellulosic dialyzers (Hemophan 25H, AFRI Medical Co., Egypt) were used. The study protocol was approved by the University of Cairo Research Committee. Written consent was obtained from each patient.

Antibody to Hepatitis-B Surface Antigen

To assess response to primary vaccination, we measured HBsAb titer eight weeks after the fourth dose using a commercially available ELISA kit (AUSAB-EIA, Abbot Labs, USA). Protection with hepatitis-B vaccination is considered to be achieved when concentration of anti-HBs antibody titers is >10 IU/L.[12,13] A nonresponse was defined as mean anti-HBs antibody titers <10 IU/L, poor responders were those with titers between 10–100 IU/L, and those with anti-HBs titers >100 IU / L were good responders.[12,13] A high response was those with titers >1,000 IU/L.[12,13]

Table 1. Patient demographic characteristics when vaccination commenced

Parameter	Mean ± SD
Age (year)	45.58 ± 10.98
Gender (male/female)	19/10
Duration of hemodialysis (year)	6.68 ± 4.93
Urea reduction ratio (%)	60.62 ± 6.96
Kt/V	1.13 ± 0.16
Serum albumin (g/dl)	3.65 ± 0.27
Hemoglobin level (g/dl)	10.04 ± 2.12
Subjective global nutritional assessment	6.24 ± 0.55
Body mass index	22.37 ± 2.85
C-reactive protein (mg/l)	9.85 ± 1.20
Ferritin (ug/l)	747.24 ± 311.68
Transferrin saturation (%)	43.44 ± 21.83

Dialysis Adequacy

We assessed dialysis adequacy using the Kt/V formula and the urea reduction ratio (URR) for all cases on three different occasions. Single-pool Kt/V (spKt/V) was assessed using the Daugirdas second-generation formula.[14] The postdialysis blood urea nitrogen (SUN) sample was obtained at the end of the dialysis session by slowing the blood pump to 50–100 ml/min for 10–20 sec, after which the blood pump was stopped and a blood sample was obtained either from the arterial bloodline sampling port or from the tubing attached to the arterial needle.[14] URR was calculated as followed and expressed as a percentage:

URR = Pre-SUN–Post-SUN/Pre-SUN[15]
(Pre-SUN is predialysis serum urea nitrogen and Post-SUN is postdialysis serum urea nitrogen)

Nutritional Status

Body mass index (BMI) was calculated as body weight in kilograms divided by height squared. Subjective global nutritional assessment (SGA) was used to evaluate overall protein-energy nutritional status before the commencement of hepatitis-B vaccination.[16] SGA includes six subjective assessments; four assessments based on the patient's history of weight change, dietary intake, gastrointestinal symptoms and functional capacity status; and two assessments based on the physician's grading of muscle wasting, presence of edema/ascites, loss of subcutaneous fat and existing chronic comorbidities, and acute metabolic stress. Based on these assessments, each patient was given a score that reflects nutritional status as follows:

1. Mild malnutrition to well nourished: 6 or 7 rating in most categories or significant, continued improvement.
2. Mild-to-moderately malnourished: 3, 4 or 5 rating, no clear indication of normal status or severe malnutrition.
3. Severely malnourished: 1 or 2 rating in most categories/significant physical signs of malnutrition.

Patients were also subjected to the following biochemical tests for nutritional assessment: predialysis serum albumin, ferritin, C-reactive protein (CRP), and percent transferrin saturation (TSAT) at the commencement of vaccination.

Statistics

The results were summarized as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was used for testing the significance of differences of the values measured between responders and nonresponders. Multiple linear regression analysis was performed to assess factors influencing response to vaccine. P value <0.05 was taken as statistically significant. All analyses were performed using the SPSS/PC+ package (SPSS Inc., Chicago, IL).

RESULTS

The patient demographic and nutritional parameters are shown in Table 1. The mean URR and Kt/V values were 60.62 ± 6.96% and 1.13 ± 0.16, respectively. Both are lower than values recommended by the National Kidney Foundation—65% and 1.2, respectively).[17] The mean SGA score and percent TSAT were 6.24 ± 0.55 and 43.44 ± 21.83%, respectively. The mean hemoglobin level was 10.04 ± 2.12 g/dl—lower than the target of 11–12 g/dl recommended by the National Kidney Foundation.[17]

Antibody Response to Vaccination

Twenty-seven patients (93.10%) responded to primary vaccination (Figure 1). Of these, seven patients (24.14%) were high responders with anti-HBs level >1,000 IU/L, 19 patients (65.51%) were good responders and had anti-HBs level in the range of 100–1000 IU/L, and one patient (3.45%) was a poor responder with anti-HBs level <100 IU/L.

Factors Affecting Antibody Response

Responders to primary vaccination had significantly higher levels of URR (%) and Kt/V compared to nonresponders (63.61 ± 6.97% and 1.25 ± 0.15 vs. 52.0 ± 2.10% and 0.92 ± 0.13, respectively, P<0.05). There was no statistically significant difference in age, gender, duration of hemodialysis therapy, hemoglobin, CRP,

Figure 1. Response rates to hepatitis-B vaccination

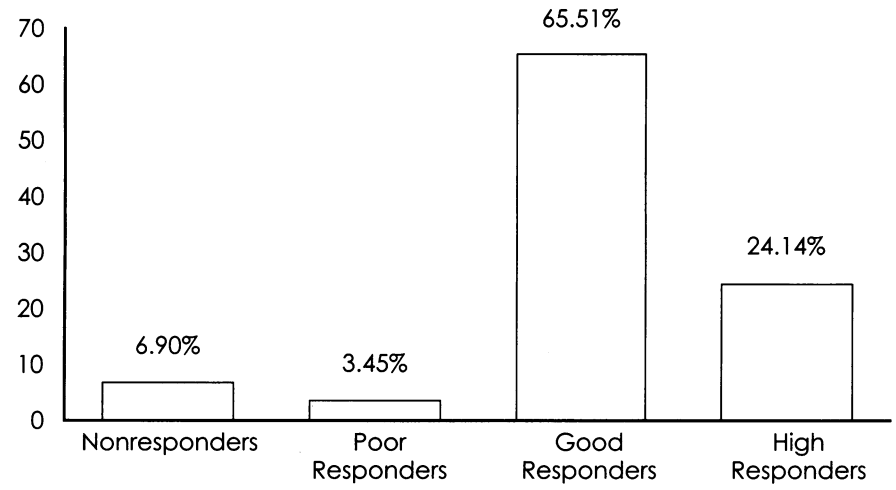

Table 2. Patient variables of responders and nonresponders to primary vaccination

Parameter	Responders (n=27)	Nonresponders (n=2)	P Value
Age	45.25 ± 11.05	50.00 ± 12.72	0.12
Gender (male/female)	1.7	2	0.09
Duration of hemodialysis	6.88 ± 5.09	4.50 ± 2.12	0.30
Urea reduction ratio (%)	63.61 ± 6.97	52 ± 2.10	0.001*
Kt/V	1.25 ± 0.15	0.92 ± 0.13	0.017*
C-reactive protein (mg/l)	8.87 ± 1.24	9.91 ± 0.58	0.34
Transferrin saturation (%)	42.70 ± 22.11	53.50 ± 20.50	0.41
Ferritin (ug/l)	756 ± 304.76	629.00 ± 524.67	0.47
Albumin (g/dl)	3.65 ± 0.29	3.70 ± 0.00	0.83
Hepatitis B (g/dl)	10.12 ± 2.17	8.85 ± 0.77	0.31
Subjective global nutritional assessment	6.24 ± 0.55	6.20 ± 0.84	0.90
Body mass index	22.07 ± 2.51	22.67 ± 3.22	0.94
Anti-HBs level (IU/L)	597.25 ± 354.40	5.5 ± 2.12	<0.00001*

TSAT, serum ferritin and albumin levels, and BMI and SGA scores between responders and nonresponders (Table 2). Multiple regression analysis showed no significant correlation between HBsAb level and subject age, duration of hemodialysis therapy, serum albumin, hemoglobin, CRP, TSAT, serum ferritin, BMI and SGA scores, URR (%) and Kt/V levels, on the other hand (Table 3).

DISCUSSION

The present study showed a very high response to hepatitis-B vaccination among hemodialysis patients. Twenty-six patients (89.65%) showed good antibody response (>100 IU/L) after the complete vaccination. Previous studies in hemodialysis patients have shown a variable hepatitis-B vaccination response rate ranged from 47–73%.[4-6] Similar, good responses to hepatitis-B vaccination in hemodialysis patients had also been observed in areas with intermediate endemicity (2–8% prevalence) of HBV, such as in Brazil, which approached 89.5% in one study.[13]

Several studies have demonstrated that hepatitis-B vaccine, even when used at high dose, does not elicit as good a response in patients with chronic renal failure as it does with the normal population.[4-6] The reasons for the poor response of hemodialysis patients to vaccination are multiple. Uremia impairs antigen presentation to and activation of T cells, and subsequent antibody production.[18]

In the present study, age, gender, duration of hemodialysis therapy, hemoglobin, TSAT, serum ferritin and albumin levels, and BMI and SGA scores did not predict response to hepatitis-B vaccine. These results are in agreement with those reported by Peces et al.,[19] Dacko et al.[11] and Tele et al.[13] Similarly, Roozbeh et al.[20] showed that age, gender, BMI and serum albumin concentration did not differ between responders and nonresponders to hepatitis-B vaccine.

Table 3. Independent determinants of anti-HBs level in hemodialysis patients by using multiple linear regression analysis

Factor	t Value	Beta	P Value
Constant	1.24		0.22
Age	0.862	0.205	0.40
Duration of HD	-0.129	-0.029	0.89
URR (%)	-0.397	-0.397	0.69
Kt/V	0.592	0.183	0.56
CRP	-1.737	-0.361	0.10
TSAT	0.544	0.120	0.59
Ferritin	1.108	0.244	0.28
Albumin	-1.181	-0.255	0.25
Hepatitis B	1.400	0.332	0.17
SGA	-0.735	-0.169	0.47
BMI	-1.822	-0.436	0.08

HD: hemodialysis; CRP: C-reactive protein; TSAT: Transferrin saturation; SGA: subjective global nutritional assessment; BMI: body mass index

In contrast, Fernandez et al.[10] had shown that malnutrition negatively influences the response to the HBV vaccine in hemodialysis patients; patients with serum albumin levels between 3–3.5 g/dl were nonresponders in a higher percentage (87.5%) than those with serum albumin levels between 4.5–5 g/dl (18.8%). Kara et al.[21] also showed that hemodialysis patients with serum albumin levels >3.5 g/dl had too much antibody response to hepatitis-B vaccine.

A recent meta-analysis of 17 clinical trials showed decreased response to hepatitis-B vaccination among older dialysis patients,[22] which might be attributed to age-associated changes in immune status. This effect was not evident in the current study, as our cohort of dialysis patients was relatively younger compared to the other studies.[4,11,23]

ESRD is associated with monocyte activation, overproduction of proinflammatory cytokines and immune dysfunction.[24] Little data exist concerning the relationship between chronic inflammation and antibody response to vaccinations in the dialysis population.[24] In the current study, no statistically significant difference in CRP level was found between responders and nonresponders. This finding may be explained by the variable counterproduction of anti-inflammatory cytokines such as interleukin-10 (IL-10), resulting in better B-cell function in uremic patients.[25,26] Patients producing higher levels of IL-10 exhibit reduced uremia and dialysis-induced chronic inflammation and respond better to vaccines.[26]

In the present study, responders to HBV vaccination had significantly higher levels of URR (%) and Kt/V compared to nonresponders (63.61 ± 6.97% and 1.25 ± 0.15 vs. 52.0 ± 2.10% and 0.92 ± 0.13, respectively, $P<0.05$). The positive effect of efficient dialysis on HBV vaccination response was also reported previously in hemodialysis patients.[27] Similarly, in a study of peritoneal dialysis patients immunized with the hepatitis-B vaccine, the initial weekly Kt/V was 2.37 and 2.01 in responders and nonresponders, respectively.[11] Efficient dialysis might lead to an enhanced response because dialysis helps to restore impaired B7-2 (CD 86) expression on monocytes of dialysis patients.[28] However, the favorable effect of efficient dialysis on immune function has not been confirmed by other investigators.[4,23,29]

Seroprevalence of HCV antibodies was reported to be high (49.1%) among ESRD patients in Egypt and was correlated significantly with blood transfusion.[30] In the current study, all patients were anti-HCV antibody positive. The status of positivity of anti-HCV did not pose a negative effect on HBV vaccination of hemodialysis patients in the present study. This was reported previously by a study from Taiwan,[31] by Peces et al.[19] and Majdan et al.[32] Others have shown that HCV infection may reduce the effectiveness of hepatitis-B vaccine in hemodialysis patients.[4,33] Twenty-three percent of HCV-infected

patients had anti-HBs of >100 IU/L in one study.[4] Effective immunity was observed in 12.5% of HCV-positive and in 35.3% of HCV-negative patients in another study.[33]

CONCLUSION

We reported a very good response to hepatitis-B vaccination among a group of HCV-positive hemodialysis patients that neither correlated with age, systemic inflammation nor nutritional status. Efficient hemodialysis was associated with good response to hepatitis-B vaccine. Future studies are needed to determine the most cost-effective vaccination regimen for hemodialysis patients in our region.

REFERENCES

1. Cheng CH, Huang CC, Leu ML, et al. Hepatitis B vaccine in hemodialysis patients with hepatitis C infection. *Vaccine.* 1997;15(12-13):1353-1357.

2. CDC. Outbreaks of Hepatitis B Virus infection Among Hemodialysis Patients-California, Nebraska, and Texas, 1994. *MMWR.* 1996;45:285-288.

3. Qirbi N, Hall AJ. Epidemiology of hepatitis B virus infection in the Middle East. *East Mediterr Health J.* 2001;7:1034-1045.

4. Navarro JF, Teruel JL, Mateos ML, et al. Antibody level after hepatitis B vaccination in hemodialysis patients: influence of hepatitis C virus infection. *Am J Nephrol.* 1996;16(2):95-97.

5. Buti M, Viladomiu L, Jardi R, et al. Long term immunogenicity and efficacy of hepatitis B vaccine in hemodialysis patients. *Am J Nephrol.* 1992;12 (3):144-147.

6. Pasko MT, Bartholomew WR, Beam TR, et al. Long term evaluation of the hepatitis B vaccine (Heptavax-B) in hemodialysis patients. *Am J Kidney Dis.* 1988;11(4): 326-331.

7. Docci D, Cipolloni P, Baldrati L, et al. Immune response to a recombinant hepatitis B vaccine in hemodialysis patients. *Int J Artif Organs.* 1990;13 (7):451-453.

8. DaRoza G, Loewen A, Djurdjev O, et al. Stage of chronic kidney disease predicts Seroconversion after hepatitis B immunization: earlier is better. *Am J Kidney Dis.* 2003;42(6):1184-1192.

9. Magnani G, Calzetti C, Campari M, et al. Immune response to hepatitis B vaccine and duration of protection in a dialysis unit. *Acta Biomed Ateneo Parmense.* 1987;58(1-2):41-47.

10. Fernandez E, Betriu MA, Gomez R, et al. Response to the hepatitis B virus vaccine in hemodialysis patients: influence of malnutrition and its importance as a risk factor for morbidity and mortality. *Nephrol Dial Transplan.* 1996;11:1559-1563.

11. Dacko C, Holley J. The influence of nutritional status, dialysis adequacy, and residual renal function on the response to hepatitis B vaccination in peritoneal dialysis patients. *Adv Perit Dial.* 1996;12:315-317.

12. Eardley K, Jones H, Osman H, et al. Efficacy of the accelerated hepatitis B vaccination schedule used in hemodialysis patients post-exposure to virus: a single centre experience. *Nephrol Dial Transplant.* 2002;17:1982-1987.

13. Tele SA, Martins RM, Lopes CL, et al. Immunogenicity of a recombinant hepatitis B vaccine in hemodialysis patients and staff. *Eur J Epidemiol.* 2001;17:145-149.

14. Daugirdas JT. Simplified equations for monitoring Kt/V, PCRn, eKt/V, and ePCRn. *Adv Ren Replace Ther.* 1995;2:295-304.

15. Daugirdas JT, Van Stone JC. Physiologic principles and urea kinetics modelling. In: Handbook of Dialysis, 3rd ed. Daugirdas JT, Blake P, Ing T, eds. Lippincott Williams & Wilkins, 2001:15-45.

16. Goldestein D. Assessment of nutritional status in renal diseases. In: Handbook of nutrition and the kidney, 3rd ed. Mitch W, Klahr S, eds. Lippincott-Raven; 1998:45-86.

17. National Kidney Foundation. K/DOQI Update 2000. www.kidney.org/professional/kdoqi/guidelines.

18. Chetanoud L, Herbelin A, Beauran G, et al. Immune deficiency of uremic patients. *Adv Nephrol.* 1990;19:259-274.

19. Peces R, de la Torre M, Alcazar R, et al. Prospective analysis of the factors influencing the antibody response to hepatitis B vaccine in hemodialysis patients. *Am J Kidney Dis.* 1997;29(2):239-245.

20. Roozbeh J, Moini M, Lankarani KB, et al. Low dose intradermal versus high dose intramuscular hepatitis B vaccination in patients on chronic hemodialysis. *ASAIO J.* 2005;51(3):242-245.

21. Kara I, Yilmaz M, Suner A, et al. The evaluation of immune responses that occur after HBV infection and HBV vaccination in hemodialysis patients. Vaccine 22:3963-3967, 2004.

22. Fabrizi F, Martin P, Dixit V, et al. Meta-analysis:the effect of age on immunological response to hepatitis B vaccine in end stage renal disease. *Aliment Pharmacol Ther.* 2004;20:1053-1062.

23. Elwell RJ, Neumann M, Bailie GR. Factors associated with long term antibody production induced by hepatitis B vaccine in patients undergoing hemodialysis:a retrospective cohort study. *Pharmacotherapy.* 2003;23 (12):1558-1563.

24. Vlassopoulos D. Hepatitis B vaccination in chronic renal failure patients. www.uninet.ed/cin2003/conf/index.html.

25. Girndt M, Kohler H, Schiedhelm-Weick E, et al. Production of interleukin-6, tumor necrosis factor _ and interleukin-10 in vitro correlates with the clinical immune defect in chronic hemodialysis patients. *Kidney Int.* 1995; 47:559-565.

26. Girndt M, Sester U, Sester M, et al. The interleukin-10 promoter genotype determines clinical immune function in hemodialysis patients. *Kidney Int.* 2001;60 (6):2385-2391.

27. Kovacic V, Sain M, Vukman V. Does efficient hemodialysis improve the response to hepatitis B virus vaccination? *Lijec Vjesn.* 2004;126(5-6):133-137.

28. Girndt M, Sester M, Sester U, et al. Defective expression of B7-2 (CD86) on monocytes of dialysis patients correlates to the uremia-associated immune defect. *Kidney Int.* 2001;59:1382-1389.

29. Weinstein T, Chagnac A, Boaz M, et al. Improved immnogenicity of a novel third generation recombinant hepatitis B vaccine in patients with end stage renal disease. *Nephron Clin Pract.* 2004;97:c67-c72.

30. Afifi A, Karim MA. Renal replacement therapy in Egypt: first annual report of the Egyptian society of nephrology. *East Mediterr Health J.* 1999;5(5):1023-1029.

31. Cheng CH, Huang CC, Leu ML, et al. Hepatitis B vaccine in hemodialysis patients with hepatitis C viral infection. *Vaccine.* 1997;15(12-13):1353-1357.

32. Majdan M, Polz M, Ksiazek A, et al. The response to the hepatitis B virus vaccine in patients undergoing hemodialysis and peritoneal dialysis-personal experience. *Przegl Lek.* 1995;52(6):307-310.

33. Muszytowski M, Manitius J, Ruszkiewicz-Folda M. Prevalence of response to anti-HBV infection in patients on maintenance hemodialysis infected with hepatitis C virus (HCV). *Przegl Lek.* 1996;53(5):417-419. ■