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## The evaluation of the effectiveness of multiple dose intradermal hepatitis B re-vaccination in hemodialyzed patients not responding to standard method of immunization

Ocena skuteczności rewakcytacji przeciw wirusowemu zapaleniu wątroby typu B wielokrotnymi śródskórnymi dawkami u pacjentów hemodializowanych nie odpowiadających na standardową immunizację

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Hepatitis B is a serious epidemiological problem in uremic patients treated with renal replacement therapy. A high proportion of hemodialyzed patients do not respond to the standard method of intramuscular (i.m.) hepatitis B vaccination. Low-dose intradermal (i.d.) inoculations and supplementary i.m. injections have been reported to improve the responsiveness in formerly non responding uremic patients. We applied a inoculation schedule of 10 µg Engerix B i.d. in 49 pts and i.m. (control group) in 13 pts once a week during 12 consecutive weeks in order to compare the effectiveness of the various ways of immunization in maintenance dialyzed patients not responding to standard vaccination. Serum anti-HBs antibody level, as well as biochemical and immunological parameters were examined. Already one month after initiation of the cycle, 57.1% of patients in the i.d. group responded by achieving the minimum protective anti-HBs antibody level (>10 IU/l.); while 14.3% reached full adequate anti-HBs antibody level (>100 IU/l.). After the full therapy period, anti-HBs antibody level >100 IU/l. was achieved in 42.9% of the patients, while a total of 81.7% of patients reached the anti-HBs antibody level >10 IU/l. In 18.4% of patients no response was observed. Surprisingly similar results were achieved in the i.m. group. Twelve months after termination of the inoculation cycle we noted decrease of anti-HBs antibody level; the values >100 IU/l. was observed only in 18.4% of the study group, while 87.8% reached a titre >10 IU/l. We found a relationship between the effectiveness of immunization and RBC count, total serum protein and albumin levels and GGTP activity. Mitogen stimulation indexes in both groups were 4-5 times lower in

Wirusowe zapalenie wątroby typu B stanowi istotny problem epidemiologiczny w populacji pacjentów dializowanych. Znaczny odsetek pacjentów leczonych hemodializami (HD) nie odpowiada na klasyczne szczepienie drogą domięśniową (i.m.). Opisywano natomiast większą skuteczność małych dawek szczepionki podanej śródskórnie (i.d.) z następowymi iniekcjami i.m. u pacjentów z niewydolnością nerek uprzednio nie odpowiadających na szczepienie. Aby ocenić skuteczność różnych metod immunizacji u chorych leczonych powtarzanymi dializami nie odpowiadającymi na standardowe szczepienie przeprowadzono je u 49 chorych według schematu 10 µg Engerix B i.d. raz w tygodniu przez okres 12 tygodni oraz i.m. (grupa kontrolna) u 13 pacjentów. W obu grupach oznaczano poziomy przeciwciał anti-HBs oraz parametry biochemiczne i immunologiczne. Już w miesiąc po rozpoczęciu szczepień 57,1% pacjentów z grupy i.d. odpowiedziało na immunizację uzyskując minimalny ochronny poziom przeciwciał anti-HBs (>10 IU/l), a 14,3% uzyskało w pełni zadowalający poziom przeciwciał anti-HBs (>100 IU/l). Po zakończeniu szczepień poziom przeciwciał anti-HBs > 100 IU/l uzyskano u 42,9% pacjentów, a poziom > 10 IU/l u 81,7%; 18,4% pacjentów nie odpowiedziało na szczepienie. Wyniki uzyskane w grupie szczepionej i.m. były podobne. W 12 miesięcy po zakończeniu cyklu szczepień obserwowano obniżenie poziomu przeciwciał anti-HBs; wartości > 100 IU/l występowały tylko u 18,4% badanych pacjentów, podczas gdy 87,8% pacjentów wykazywało poziom anti-HBs > 10 IU/l. Stwierdzono zależność pomiędzy skutecznością szczepień a liczbą erytrocytów, poziomami białka całkowitego i albumin oraz aktywnością GGTP. Wskaźniki stymulacji mitogenami w

comparison to reference values in the general population. In the study group that did not respond to vaccination, mitogen stimulation indexes were 2 times lower as compared to the group characterized as having a good response. In conclusion, the route of injection seems to be less important than the frequency and number of doses of the vaccine. Anemia and malnutrition may be responsible for the worse response to vaccination against hepatitis B virus.

### Introduction

HBV and HCV are well known epidemiological problems in Poland. Uremic patients treated with maintenance hemodialysis are exposed to hematogenous hepatic viral infections [20,33]. Multiple blood transfusions, hemodialysis procedure and frequent intravenous injections are the main conductive factors. Currently, the risk of HBV infection has decreased due to the administration of recombinant erythropoietin instead of blood transfusions in chronic anemia treatment, strict sanitary regulations in dialysis units and an inoculation program introduced in all hemodialyzed patients [12,58]. Effective vaccination plays an essential role in the prevention of hepatitis B. Unfortunately, only 60-80% of hemodialysis patients produce protective (>10 IU/l) level of anti-HBs antibodies after administration of a double dose (40 µg) of recombinant vaccine in 0-1-2-6 months schedule [13,25,31,45]. Poor response to vaccination and fast decrease of specific antibody titers is the result of immunological system dysfunction, commonly coexisting with end-stage renal failure (ESRF). Dysfunction concerns both humoral and cellular responses [10,34,42,57]. The production of specific antibodies after antigenic stimulation is low although levels of IgG, IgM and IgA in the serum of dialysis patients are usually normal. Disorders in the T-cell and B-cell interactions, as well as impaired antigen presentation are also affirmed. Paradoxically, deficiency of immunological response coexists with laboratory signs of chronic stimulation of the immune system which is probably due to repeated dialysis sessions and contact of blood with the artificial dialysis membrane [27,36,51]. Frequent polyetiological infections are observed in the population of dialysis patients. The course of infection is usually more dynamic and complicated than in the general population [5,6,11]. Malignant neoplasmas are more frequent in this group of patients [64]. The activity of some autoimmune diseases decreases during hemodialysis treatment [44]. The reasons of immune disorders are primary kidney diseases, immunosuppressive treatment in the past, effects of uremic toxins, iron overload, malnutrition, hypovitaminosis and microelements deficiency [47]. Very important is also the method of renal replacement therapy and biocompatibility of materials used in dialysis equipment [38,51,60]. Acquired immune depletion changes also the clinical image of hepatitis B. Acute forms are very rare, usually the symptoms are scanty and non-characteristic. The complete elimination of HBs antigen is difficult or impossible and in 40-60% of cases disease transforms into

obu grupach były 4-5 razy niższe w stosunku do wartości referencyjnych w ogólnej populacji. W grupie pacjentów, którzy nie odpowiedzieli na szczepienie wskaźniki powyższe były 2-krotnie niższe niż w grupie pacjentów, u których uzyskano dobrą odpowiedź. Wnioski: Droga podania wydaje się być mniej istotna niż częstość i ilość podanych dawek szczepionki. Niedożywienie i niedokrwistość mogą być odpowiedzialne za gorszą odpowiedź na szczepienie przeciwko wirusowemu zapaleniu wątroby typu B.

chronic liver inflammation [32,39]. Additional problems appear after kidney transplantation because immunosuppressive drugs reactivate and intensify the replication of HBV, which may lead to many complications as fulminant hepatitis or progression into hepatic cirrhosis [1,33]. Achievement of protective anti-HBs antibody levels in hemodialyzed patients, non-responding to the standard method of immunization, is therefore very important and it was the aim of many clinical trials. The type of vaccine, its dose, inoculation route, number of injections were considered in those studies [21, 24,55,62]. Some researchers tried to use adjuvants as GM-CSF or oral immunomodulators like levamisole [3, 15, 18, 22]. The last few years, hope was put into intradermal (i.d.), low dose and short-period multi-injection vaccination mode. This method seems to be more effective, safer and cheaper than the standard double dose intramuscular scheme. Until now the literature data is ambiguous and the effectiveness of intradermal vaccination protocol needs ultimate confirmation [50,65].

The aim of the study was to evaluate the effectiveness of alternative intramuscular (i.m.) and intradermal methods of re-vaccination in hemodialyzed patients not responding to standard inoculation schedule.

### Patients and Methods

The study was performed in 62 uremic patients, treated with maintenance hemodialysis at the Nephrology Clinic of the Jagiellonian University Hospital in Kraków, Nephrology Departments of the Saint Luca's Province Hospital in Tarnów and E. Szczekliki's Hospital in Tarnów. There were 24 females and 38 males, with mean age 56,1 years (range 22-78). The end-stage renal failure of the patients was due to chronic glomerulonephritis (18%), diabetic nephropathy (14,5%), chronic interstitial nephritis (10,5%), polycystic kidney disease (5%), kidney cancer (3,3%), amyloidosis (1,6%) and lupus nephritis (1,6%). In 45% of cases the exact etiology of renal failure was unknown. The most common coexisting diseases were hypertension (87%), coronary heart disease (54,8%) and diabetes (9,6%). Anti-HCV antibodies and HCV-RNA were positive in 7 patients. The median duration of hemodialysis treatment was 23 months (range 1-118 months). Routine, 4 hour long bicarbonate hemodialyses were performed three times a week. In the studied group there were 13 patients treated with peritoneal dialysis in the past. Erythropoietin was administered in 61 patients due to the anaemia.

The protocol was approved by the Bioethics Committee of the Jagiellonian University.

The patients were informed and gave their consent for all performed procedures.

### Study protocol

All chosen subjects did not respond to standard, double dose (40 µg), 0-1-2-6 months i.m. vaccination protocol with Engerix B (SmithKline Beecham). The serum anti-HBs antibody level in these patients was less than 9 IU/l in a period of 1 to 6 months after completion of the immunization. Forty nine patients were vaccina-

ted i.d. applying 10 µg of Engerix B vaccine (1/2 ampoule), once a week during 12 consecutive weeks. Serum anti-HBs antibody level was controlled monthly for 6 months after vaccination cycle initiation. In cases where serum anti-HBs antibody level reached 100 IU/l or more, further vaccination was terminated and these patients were considered as having reached protective antibody level. Those patients, who did not achieve sufficient anti-HBs antibody level (>10 IU/l), received an additional reminder dose (10 mg), maximum twice. Dynamics of the immune response was also evaluated by controlling the serum anti-HBs antibody levels 9 and 12 months after termination of the vaccination cycle. The control group was comprised of 13 patients inoculated i.m. with the same number and dose of Engerix B vaccine at identical time intervals. Immunization effectiveness was evaluated using the following scale: minimum adequate anti-HBs antibody level (>10 IU/l), middle anti-HBs antibody level (>30 IU/l), and full, adequate anti-HBs antibody level (>100 IU/l).

### Serum examinations

Presence of HBs antigen, anti-HBs, anti-HBc, anti-HCV antibody titers were measured by ELISA method (Hepatodiagnostika Uniform II). Aside from hepatic serological tests, all patients of the i.d. and i.m. groups were tested every month for serum levels of bilirubin, activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and cholinesterase, blood cell count and dialysis adequacy indicators (Kt/V, pcr, TAC) calculated with the computer program Nephron for Windows. All patients were also tested every two months for serum level of albumin and total protein, every six months for level of parathormone (PTH). Once, at the beginning of the study immunoglobulins IgG, IgM and IgA was determined.

### Immunologic studies

10 patients were qualified for immunologic diagnostics: 5 responded quickly after a few doses of vaccine with full immunization (anti-HBs > 100 IU/l), and 5 did not respond after 12 routine and two additional reminder injections.

### T-lymphocyte response in vitro

Mononuclear leukocytes were isolated from EDTA-blood by standard Ficoll-Paque (Pharmacia, Uppsala, Sweden) centrifugation. Obtained lymphocytes were cultured on RPMI 1640 medium (Gibco, Paisley, GB) with 10% fetal calf serum (FCS, Biochrom, Germany) and antibiotics (penicillin 100 IU/ml, streptomycin 100 µg/ml, Polfa, Poland). The cells were cultured in 96-well plates (0.2 ml per well) for 72 hours in a 5% CO<sub>2</sub> atmosphere. In a proportion of cultures the cells were stimulated by mitogens: PHA (2.5 µg/ml, Wellcome, GB), PWM (final dilution 1:100, Gibco) and anti-CD3 antibody (1 µg/ml, Immunotech, Marseille, France). For the final 8 hours to each well 10 µl of 3H thymidine (5 µCi/mM, Amersham, GB) was added. Cells were harvested on a glass fiber filter and 3H-thymidine radioactivity was evaluated in a scintillation counter (Beckmann-Dickinson, Lier, Norway). The stimulation index was defined as proportion of the radioactivity of mitogen-stimulated cells to non-stimulated.

### T-cell subpopulations

The cells obtained from centrifugation were twice in PBS and counted, followed by immunostaining with monoclonal mouse antibodies raised against CD3, CD4, CD8, HLA-DR, CD19, CD16+56 (Becton-Dickinson, Heidelberg, Germany) conjugated in combinations with fluorescein isothiocyanate (FITC) and phycoerythrin (RPE). The labeled cells were evaluated by flow cytometry utilising FACScan (Becton-Dickinson, Heidelberg,

Germany). In each sample 5000 cells were analyzed.

**Vaccine side-effects**

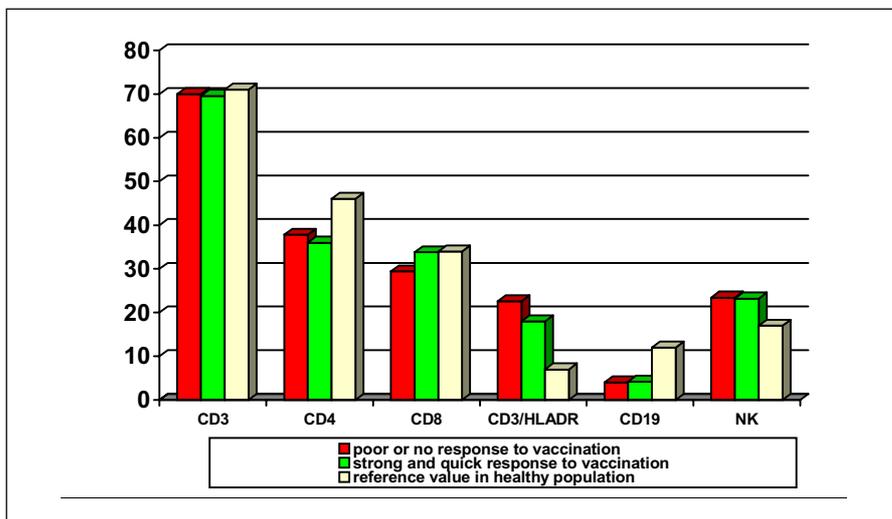
The presence of vaccine side-effects was monitored in all patients. All patients were asked to report the presence of any related to the injection symptoms during 3 following days after every inoculation.

**Statistical analysis**

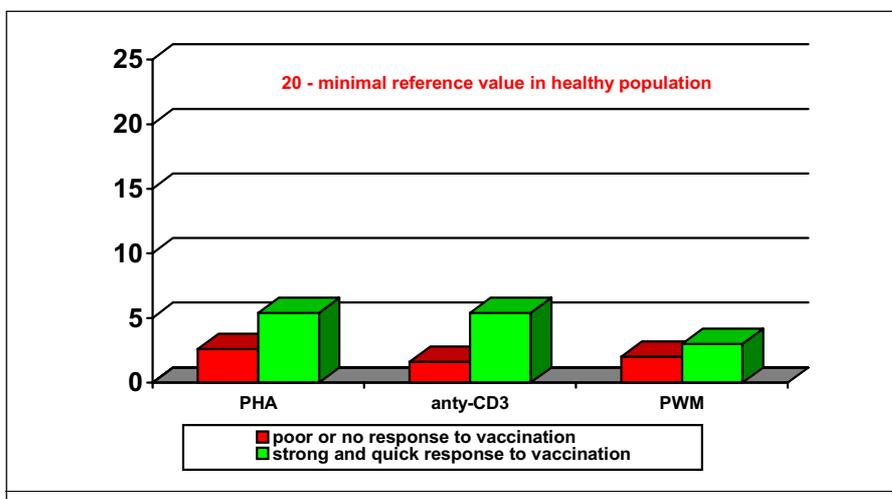
For data management and statistical analysis, Statistica 5.1 ('97 Edition, StatSoft, Inc.) software was used. The comparison between groups was performed using the Mann-Whitney U-test. Significances between examined variables were calculated with Spearman test.

**Results**

Vaccine site effects were not noted in any patients. One month after initiation of the cycle, 57.1% patients in the i.d. group responded by achieving the minimum protective anti-HBs antibody level (>10 IU/l.), while 36.7% achieved the middle anti-HBs antibody level (>30 IU/l.), and 14.3% reached full adequate anti-HBs antibody level (>100 IU/l.). Adequate anti-HBs antibody level after full therapy period (>100 IU/l.) was achieved in 42.9% of the patients, while a total of 81.7% of patients reached the anti-HBs antibody level >10 IU/l. In 18.4% of patients no response was observed. Nine months after the vaccination cycle initiation, only 22.4% of the study group maintained the anti-HBs antibody level >100 IU/l., 51% attained >30 IU/l., while a total of 83.6% of patients were protected. A further decrease of anti-HBs antibody level was noted after 12 month after termination date of the vaccination cycle. The level of >100 IU/l. was observed only in 18.4% of the study group, 36.7% attained a level of >30 IU/l., while 87.8% reached a level of >10 IU/l.). Reminder doses (10 µg) were administered to 15 (30.6%) patients who presented antibody level below 10 IU/l. Anti-HBs antibodies titres, achieved in the group vaccinated i.d. are shown in Table 1. Surprisingly good results were achieved in



**Figure 1**  
Percentage of lymphocyte sub-population in both examined groups of patients.  
Procent subpopulacji limfocytów w obu badanych grupach pacjentów.



**Figure 2**  
Mitogen stimulation indexes in both examined groups of patients.  
Indeksy stymulacji mitogenem w obu badanych grupach pacjentów.

**Table I**

**Numerical and percentage distribution of patients due to achieved anti-HBs antibody levels during intradermal immunization.**

Rozkład pacjentów w zależności od osiągniętych poziomów anty-HBs w czasie i po cyklach immunizacji w grupie szczepionych drogą śródskórną.

Anti-HBs antibodies titre (IU/l)	I month	II month	III month	IV month	V month	VI month	IX month after the termination of vaccination route	XII month after the termination of vaccination route
0-10	21 (42.9%)	13 (26.5%)	9 (18.4%)	6 (12.2%)	7 (14.3%)	6 (12.2%)	8 (16.3%)	6 (12.2%)
10-30	3 (6.1%)	3 (6.1%)	4 (8.2%)	6 (12.2%)	7 (14.3%)	8 (16.3%)	5 (10.2%)	16 (32.7%)
30-100	18 (36.7%)	12 (24.5%)	15 (30.6%)	19 (38.8%)	20 (40.8%)	22 (44.9%)	25 (51.0%)	18 (36.7%)
>100	7 (14.3%)	21 (42.9%)	21 (42.9%)	18 (36.7%)	15 (30.6%)	13 (26.5%)	11 (22.4%)	9 (18.4%)

**Table II**

**Numerical and percentage distribution of patients due to achieved anti-HBs antibody levels during intramuscular immunization (control group).**

Rozkład pacjentów w zależności od osiągniętych poziomów anty-HBs w czasie i po cyklach immunizacji w grupie szczepionych drogą domięśniową (grupa kontrolna).

Anti-HBs antibodies titre (IU/l)	I month	II month	III month	IV month	V month	VI month	IX month after the termination of vaccination route	XII month after the termination of vaccination route
0-10	4 (30.8%)	2 (15.4%)	2 (15.4%)	2 (15.4%)	0 (0%)	2 (15.4%)	1 (7.7%)	4 (30.8%)
10-30	1 (7.7%)	1 (7.7%)	1 (7.7%)	1 (7.7%)	4 (30.8%)	3 (23.1%)	3 (23.1%)	3 (23.1%)
30-100	6 (46.2%)	4 (30.8%)	2 (15.4%)	5 (38.5%)	6 (46.2%)	6 (46.2%)	8 (61.5%)	5 (38.5%)
>100	2 (15.4%)	6 (46.2%)	8 (61.5%)	5 (38.5%)	3 (23.1%)	2 (15.4%)	1 (7.7%)	1 (7.7%)

the control group (13 patients) where vaccine administration was i.m. (Table 2). Similar antibody levels were achieved in the first three months after vaccination cycle initia-

tion in both groups, even a slight increase was noted in the group vaccinated i.m. as compared to the group vaccinated i.d. The percentage of patients that had the best re-

sponse (value exceeding 100 IU/l.) was higher in the consecutive 4 months, respectively 15.4%, 46.2%, 61.5%, 38.5% (Table 2). Long term effectiveness of the i.m. group

**Table III**

**Mean anti-HBs antibody titers achieved during immunization in both examined group of patients.**

Srednie miano anty-HBs osiagnane w czasie immunizacji w badanych grupach.

Mean anti-HBs antibodies titre (IU/l)	I month	II month	III month	IV month	V month	VI month	IX month after the termination of vaccination route	XII month after the termination of vaccination route
Group vaccinated intradermal	38.54	55.66	62.97	63.98	61.82	58.76	53.08	41.86
Group vaccinated intramuscular	45.33	71.97	75.42	70.0	64.19	52.19	45.95	35.78
Both groups	39.96	59.08	65.58	65.24	62.32	57.38	51.59	40.59
	2 (15.4%)	6 (46.2%)	8 (61.5%)	5 (38.5%)	3 (23.1%)	2 (15.4%)	1 (7.7%)	1 (7.7%)

**Table IV**

**Correlation coefficient values between the response to vaccination (anty-HBV antibody titers) and red blood cell indexes.**

Współczynniki korelacji pomiędzy odpowiedzią na szczepienie (miano anty-HBV) a wartościami erytrocytów.

	I month	II month	III month	IV month
Hematocrite	r=0.35; p=0.006	r=0.33; p=0.008	r=0.3; p=0.01	r=0.25; p=0.04
Red blood cells amount	r=0.35; p=0.006	r=0.34; p=0.007	r=0.32; p=0.01	r=0.26; p=0.03
Hemoglobin	r=0.29; p=0.02	r=0.26; p=0.04	r=0.28; p=0.02	

**Table V**

**Correlation coefficient values between the response to vaccination and the level of total serum protein.**

Współczynniki korelacji pomiędzy odpowiedzią na szczepienie a poziomem białka całkowitego.

	I month	IV month	V month	VI month	IX month month after the termination of vaccination route	XII month month after the termination of vaccination route
Total serum protein	r=0.25; p=0.04	r=0.28; p=0.02	r=0.32; p=0.01	r=0.36; p=0.004	r=0.33; p=0.008	r=0.33; p=0.008

was worse when compared to the i.d. method, although no statistically relevant differences were noted (p=0.2). Twelve months after the termination of the vaccination cycle, 4 patients (30.8%) did not attain an adequate anti-HBs antibody level, in 3 (23.1%) minimal anti-HBs antibody level (10 IU/l.) was achieved, in 5 patients (38.5%) anti-HBs antibody level exceeded 30 IU/l. Anti-HBs antibody level >100 IU/l. was noted in 1 (7.7%) of the vaccination population. Anti-HBs antibody titers, achieved in i.m. group are shown in the Table 2. There was no significant difference between i.d. and i.m. patients with regard to the sero-protection rate. The mean anti-HBs antibody titers, achieved in both groups during vaccination route are illustrated in Table 3.

Positive correlation was noted between values of the red blood cell population condition and attained anti-HBs antibody levels in the first 4 months of vaccination. A relative difference between these parameters was found depending on the attained antibody level (Table 4). Better response to vaccination also correlated with the total serum protein level in the 1st, 4th, 5th, 6th, 9th, 12th month (Table 5). Total protein and albumin levels were also significantly higher in groups that attained anti-HBs antibody levels >10 IU/l. and > 30 IU/l. in comparison to patients whose antibody levels were lower than the above mentioned. GGTP activity was significantly lower in the patient groups with anti-HBs antibody levels >10 IU/l. and > 30 IU/l. than in the group with anti-HBs antibody levels <10 IU/l. and < 30 IU/l. in particular months after termination of the vaccination cycle. In the 2nd month after inoculation cycle initiation, TIBC value was significantly lower in the patient group whose anti-HBs antibody level was >10 IU/l. than in the group with antibody level <10 IU/l.

There were no significant differences in IgG, IgM, IgA serum levels in examined

hemodialyzed patients in comparison to general population.

In immunological tests no abnormalities were found in the T lymphocyte population and their sub-population and CD4/CD8 ratio was not reversed. A decrease in the percentage of lymphocyte B (CD19) was noted in peripheral blood, as well as signs of chronic stimulation of the immune system (reported as increased number of CD3 HLA-DR+ cells and NK cells) in both examined groups (Figure 1). Mitogen stimulation indexes in both groups were 4-5 times lower in comparison to reference values in general population. In the study group that did not respond to vaccination, mitogen stimulation indexes were 2 times lower as compared to the group characterized as having a good response (Figure 2).

#### Discussion

In the current study we illustrated results of vaccination against HBV infection, administered intradermally in a population of hemodialyzed patients. The i.d. multiple inoculation in short time intervals was effective in dialyzed patients who did not respond to standard (0-1-2-6) double dose (40 µg) method. Similar results were reported by Fabrizi et al [19, 21], Chang et al. [8] and Poux et al. [49] who applied similar immunization schedule. This method allows to obtain dynamic increase of anti-HBs antibody level and could be useful in patients requiring quick seroprotection e.g. before planned surgical operation or in post-expositional prophylaxis. However, we observed in many subjects that level of anti-HBs antibodies dramatically felt down in about 9 months after completion of inoculation cycle. In this group of patients we suggest frequent monitoring of anti-HBs levels and administration of reminding doses in cases requiring additional protection. Recent publications report that i.d. vaccination route is more efficient

than the standard i.m. method [9,10, 49]. Investigations performed on general population revealed that i.d. method of immunization allows to diminish doses of applied vaccines to reach sufficient antibody level [30]. Moreover, the i.d. method was shown to be effective in patient who did not respond to routine intramuscular vaccination. Many authors report an advantage of this method in uremic patient undergoing renal replacement therapy [30,41]. Longer exposition of HBs antigen to immune system and more numerous antigen presenting cells in the skin are believed to be the most probable explanations of this phenomenon. In our study we evaluate whether effect of vaccination depends only on a way of administration or also on number of doses and time intervals between injections. Results of immunization in the control group (vaccinated i.m.) were similar to those in i.d. vaccinated. Perhaps effectiveness of the vaccination is more associated with the amount of vaccine dosage administered (which is three times higher than in the standard method) and time interval of administration (short time interval between vaccine administration, not longer than 7 days apart) rather than the route of administration. Our findings are in agreement with the results of Bommer et al. [7] who proved that in a group of patients not responding to the standard method, at least 5 doses of vaccine are necessary to obtain a satisfactory level of protection. Short intervals between doses increase the percentage of seroconversion even in patients suffering from liver failure [14]. We suspect, it is due to longer exposition of the antigen and allows almost continuous stimulation of the immune system.

Multiple factors potentially influencing the level of the immune response for HBs vaccination were analyzed. We found statistically significant correlations between immunization response and parameters of

the red blood system (hematocrite, RBC count, hemoglobin level).

Up to date there are no convincing pathophysiological proofs for existence of indirect influence of the RBC system to the quality of immunologic response. However, Mc Faul et al [40] observed *in vitro* increased secretion of proinflammatory cytokines as IL-6, IL-8 and TNF $\alpha$  by mononuclear leukocytes after stimulation by purified hemoglobin. Low secretion of IL-2 was reported in anemic patients and its level was highly correlated with hemoglobin concentration [26]. The phagocytic activity of granulocytes was affected by low hematocrite [61]. Severe anemia may impair function of the immunologic system by hypoxia and activation of the alternate non-oxygen metabolic pathways [16].

Administration of human recombinant erythropoietin (rHu-EPO) plays the main role in the treatment of anemia in ESRD patients. Beside erythropoiesis stimulation, it influences cellular and humoral immunologic responses. Erythropoietin applied for 6 months in long-term maintenance HD patients caused increase in number of CD4+/CD4RA (T helper cells) and depletion of the amount of CD3+HLA-DR+ that are usually elevated in hemodialysis patients (59). In our study we also found increased number of CD3+HLA-DR+ lymphocytes in examined dialyzed patients. After 8 months-long rHu-EPO treatment in stable HD patients, Kaneko et al. [35] observed increase in the proportions of CD4+, HLA-DR+ cells and CD4+/CD8+ ratios that are characteristic to helper subset and activated T lymphocytes. Moreover, EPO therapy may improve proliferation indexes of cultured T lymphocytes after mitogen stimulations [53,66]. Bartunkova et al. suggest improved oxygen metabolism in rHu-EPO treated HD patients to be reasonable for better immune response rather than indirect specific influence of EPO on lymphocytes [4]. Prolonged rHu-EPO treatment improves also immunoglobulin production in HD subjects [48,52]. Anandh et al. [2] revealed significantly better response to hepatitis B immunization in the group of hemodialyzed patients treated with rHu-EPO. In our study almost all patients received rHu-EPO implementation. Different conditions of RBC system in these patients could be explained by gradual resistance to erythropoietin. Erythropoietin resistance seems to be the main reason of chronic disease anemia such as autoimmune disorders, neoplastic diseases and chronic infections. It may be elucidated by the prolonged inflammation process with secretion of multiple cytokines such as IL-1, IL-6, TNF alfa, INF gamma that impair erythropoiesis and iron metabolism, decrease of endogenous EPO synthesis and development of rHu-EPO resistance [43,17]. Higher concentrations (8-10 times) of proinflammatory cytokines are observed in hemo-dialyzed patients. The state of such "chronic activation" and "immune system fatigue" leads to weaker response to antigens [46,54]. It may be concluded that there is a common pathomechanism of rHu-EPO resistance and worse effects of administered vaccinations.

In our studies we found that patients with high TIBC were worse responders for inoculation procedure. It is in agreement with authors that reported the role of iron overload in impairments of immunity processes (56).

We found a significant relation between total protein and albumin serum concentrations and a quality of vaccination response ( $p < 0.05$ ). Malnutrition and high rate of catabolism are serious problems in patients with end stage renal failure. Uremia and dialysis procedures as well as inadequate dialysis dose intensify the catabolism processes and loss of proteins. All mentioned factors are known to seriously impair the condition of the immune system [20,34,54].

We have seen also reciprocal relationship between immunization progress and GGTP activity which may be a confirmation of an influence of liver efficiency to immune response [14].

Disability of immune system to produce protective level of anti-HBs antibodies may be due to cellular mechanisms defects [63]. In our study we did not observe depletion in lymphocyte number in peripheral blood, although some authors report moderate lymphopenia in this group of patients [36]. Percentage of T-lymphocytes and CD4/CD8 ratio obtained in our experiment resembled values in general population. Contrary, some authors report up to 50% reduction of lymphocyte T number and low CD4/CD8 ratio in uremic patients [42]. We noted decreased number of B-lymphocytes coexisting with normal serum immunoglobulin level which is in agreement with Fernandez et al. [23]. The obtained high proportions of natural killers cells and lymphocytes CD3/HLA-DR+ may reflect chronic activation of cellular response processes reported also by Girndt et al [28,29]. Low proliferation indexes on mitogen stimulation (4-5 fold lower than in general population) observed in our study appear to be a proof to cellular response dysfunction [37]. However, these indexes were approximately twice higher in a subgroup of subjects that well responded to modified multiple dose short interval method of hepatitis B inoculation. Poor or no-response to vaccination appear to be due to different degrees of the inflammation process which is supposed to damage the immune system, or to the genetical predisposition of such a group of patients.

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