

## ANTIBODY RESPONSE TO HEAT-INACTIVATED HEPATITIS B VACCINE (CLB-3 $\mu$ g) IN HEMODIALYSIS PATIENTS AND OCCUPATIONAL RISK PERSONNEL: A ONE YEAR FOLLOW-UP

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### S U M M A R Y

Immune response against hepatitis B vaccine (CLB-3 $\mu$ g) was evaluated in 59 hemodialysis patients and 20 occupational risk personnel. Seroconversion was induced in 52.5% and 70.0% respectively. Twelve months after the first dose, 37.5% of patients and 60.0% of occupational risk personnel had detectable anti-HBs level. Antibody level was expressed in sample ratio units (SRU). Considering only the responders, in the patients group 38.7% had a low anti-HBs response (2.1-9.9 SRU) 32.3% a medium response (10-99.9 SRU) and 29.0% a high response (>100 SRU) while in occupational risk personnel these values were 14.3%, 64.3% and 21.4% respectively. The authors suggest the use of HBV vaccines with more elevated HBsAg concentration or a reinforced immunization schedule to improve the anti-HBs response not only for patients but also for healthy persons.

**KEY WORDS:** Hepatitis B vaccine; Immune Response; Hemodialysis.

### I N T R O D U C T I O N

The prophylaxis of the hepatitis B infection by vaccination has been a major breakthrough in the control of this disease.

The use of vaccine is recommended in hemodialysis units because of the high risk of dissemination of hepatitis B virus (HBV) due to the high frequency of chronic carriers among patients with renal failure (13).

Several studies have demonstrated the efficacy of different plasma derived vaccines (Hevac B, Pasteur; Heptavax, MSD) in the prevention of hepatitis B in hemodialysis centers<sup>6, 7</sup> and among high risk groups such as children living in endemic areas<sup>17</sup> and male homosexuals<sup>18</sup>.

In 1981, BRUMMELHUIS et al.<sup>2</sup> described a simplified method of preparation of heat-inactivated hepatitis B vaccine (CLB, Netherlands). The efficacy and immunogenicity of this vaccine were demonstrated in healthy volunteers at low risk<sup>15</sup> and in some high risk groups<sup>5, 8</sup>.

This paper describes the evaluation of the immune response in hemodialysis patients and occupational risk personnel against CLB-3 $\mu$ g vaccine.

### M A T E R I A L S A N D M E T H O D S

**Vaccine:** The CLB vaccine contains 22nm particles of HBsAg of two different subtypes, ad

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and ay, purified by separation methods used for isolation of plasma components. The vaccine is heat inactivated, absorbed to aluminium phosphate and the final suspension contains 3 $\mu$ g of HBsAg per dose. This vaccine was developed by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), and was kindly provided to be evaluated in our hemodialysis patients and occupational risk personnel. The vaccine was kept at 4°C in our laboratory until issued for inoculation.

**Participants:** The vaccine was tested in a total of 59 patients (38 males) at 5 hemodialysis centers. The mean age was 41  $\pm$  13 years and 38% had been submitted to hemodialysis for more than one year. Twenty occupational risk personnel (8 males) with a mean age of 28  $\pm$  8 were also vaccinated.

All participants were volunteers and only those who were negative for any HBV marker were selected for vaccination. After the beginning of vaccination, blood specimens were obtained from all subjects at monthly intervals during a period of one year to be tested for HBsAg and anti HBs.

**Immunization protocol:** Three vaccine doses of 3 $\mu$ g were administered at one month intervals by intramuscular injection in occupational risk personnel. In hemodialysis patients the vaccine was administered in four doses of 3 $\mu$ g, given at 0, 1, 2 and 5 months. The site of injection was the deltoid region.

**Laboratory methods:** HBsAg was tested by enzyme immunoassay with reagents prepared

in our laboratory<sup>3</sup>. Anti-HBc and anti-HBs were performed using solid phase radioimmunoassay (Abbott Laboratories, USA). The level of anti-HBs was expressed in sample ratio units (SRU), which was calculated by the counts per minute of the sample over the mean counts per minute of 7 negative controls. An anti-HBs level greater than 2.1 SRU in at least two sequential samples was considered a seroconversion. A low response was considered an antibody level between 2.1 and 9.9 SRU; a medium response between 10 and 99.9 SRU and a high response equal or over 100 SRU.

**Statistical methods:** The chi-square test was used to compare the frequency of anti-HBs in both groups studied ( $\alpha = 0.05$ ).

## RESULTS

The anti-HBs seroconversion induced by hepatitis B vaccine was detected in 52.5% of hemodialysis patients and in 70.0% of occupational risk personnel. This difference was not statistically significant. Among patients, 20.3% had a low antibody response (geometric mean titer, GMT = 6.8), 16.9% a medium response (GMT = 40.0), and 15.3% a high response (GMT = 393.0), considering the maximum antibody level developed. The remaining 47.5% did not show any anti-HBs response during the one year follow-up. Among occupational risk personnel, 10.0% had low response (GMT = 6.2), 45.0% a medium response (GMT = 28.0), 15.0% a high response (GMT = 493.5) and 30.0% failed to respond to the vaccine. These results are shown in figure 1.

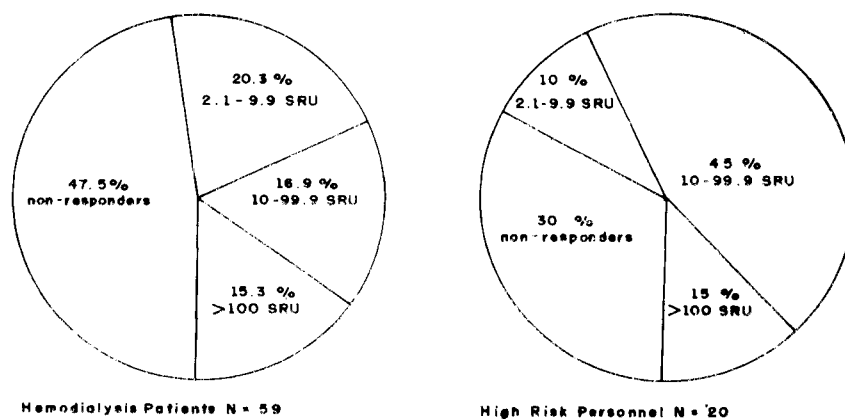


Fig. 1 — Anti-HBs response in SRU in hemodialysis patients and occupational risk personnel.

Considering only the responders, in the patients group, the vaccine induced a low response in 38.7%, a medium response in 32.3% and a high response in 29.0%, while in occupational risk personnel, these values were 14.3%, 64.3% and 21.4% respectively.

In the patients group, the percentage of anti-HBs responders decreased with age. In those under 15 years old, 75.0% responded to the vaccine a proportion which decreased gradually to 14.0% in those aged over 61 years old (Fig. 2).

The time of appearance of anti-HBs in patients varied from 1 to 9 months after the beginning of vaccination; 64.5% of the responders

developed anti-HBs within the first three months after the first dose; 12.9% developed between the third and fifth month when the fourth dose of vaccine (day 150) was administered and a further 22.6% became anti-HBs positive after the fifth month. Although the maximum anti-HBs response showed a wide variation in 67.7% it always occurred after the fourth dose (fifth month). The cumulative frequency of anti-HBs appearance and maximum response related to time of vaccination are shown in fig. 3. In the occupational risk group, antibody developed over a shorter period, from 1 to 3 months after the first dose, and 85.7% of them showed maximum response between 3 and 6 months (Fig. 3).

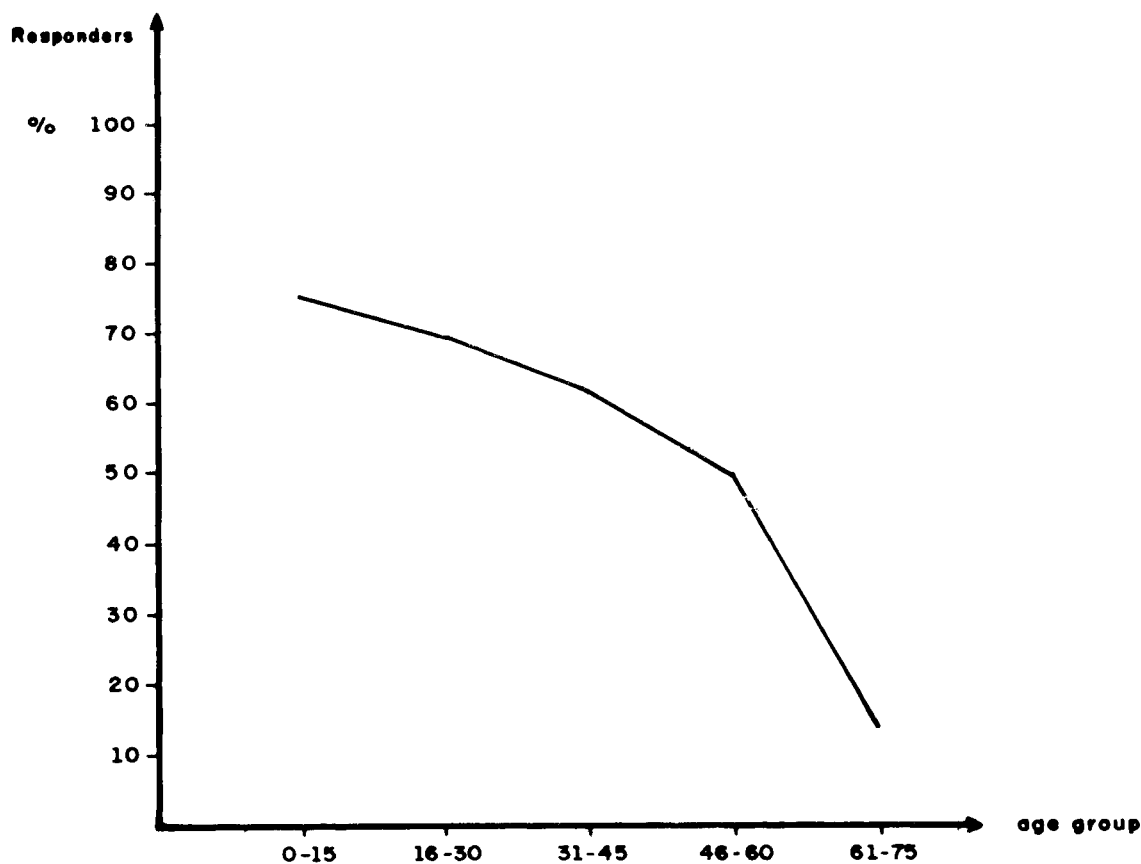


Fig. 2 — Antibody response to Hepatitis B vaccine in hemodialysis patients related to the age.

Twelve months after the first dose, anti-HBs level decreased in both groups. In the patients group, out of 28 responders, only 21 (75.0%) still had antibody at this time, excluding 3 patients

who died. In the group of high responders all remained positive, but the level of anti-HBs decreased markedly. Among 10 medium responders, 3 became negative in the period of the fo-

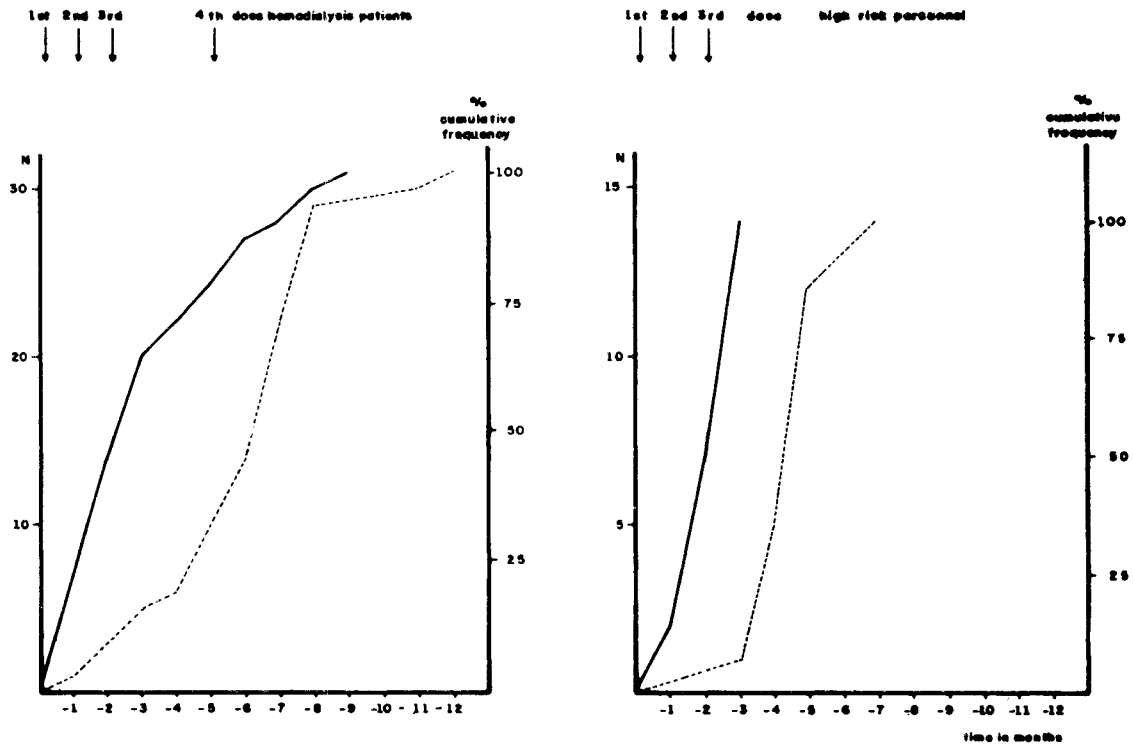


Fig. 3 — Anti-HBs response in patients and occupational risk personnel related to time of vaccination (CLB-3 $\mu$ g) considering only the responders.

low-up and the antibody level also decreased in the remaining 7 responders. From 12 patients who had low response, four became negative, one died and the remaining 7 had very low antibody levels after one year (Table I).

In the occupational risk personnel, all high and medium responders remained positive after the one year follow-up and only two who had developed low titers became negative during the period of study (Table I).

TABLE I  
Decrease after 12 months of anti-HBs response to hepatitis B vaccine (CLB-3 $\mu$ g) in hemodialysis patients and occupational risk personnel

SRU	Hemodialysis patients						Occupational risk personnel					
	Maximum response			12 mo. after 1st dose			Maximum response			12 mo. after 1st dose		
	N	(%)	GMT	N	(%)	GMT	N	(%)	GMT	N	(%)	GMT
2.1 — 9.9	12/59	(20.3)	6.8	7*/56	(12.5)	3.2	2/20	(10.0)	6.2	0/20	(0)	0
10 — 99.9	10/59	(16.9)	40.0	7/56	(12.5)	11.3	9/2	(45.0)	28.0	9/20	(45.0)	5.5
> 100	9/59	(15.3)	393.0	7**/56	(12.5)	32.7	3/20	(15.0)	493.5	1/20	(5.0)	152.5
	31/59	(52.5)		21/56	(37.5)		14/20	(70.0)		12/20	(60.0)	

\* 1: patient died

\*\* 2: patients died

## DISCUSSION

In general, antibody response in patients with chronic renal failure is lower than in healthy persons<sup>16</sup>. Nevertheless, a high degree of protection against hepatitis B infection has been reported in several trials of HBV vaccine carried out in some groups at risk, including hemodialysis patients<sup>6, 7, 17, 18</sup>. Studies with CLB-3 $\mu$ g vaccine, have demonstrated seroconversion rates ranging from 60 to 88% in such patients<sup>11, 16</sup>. In the present study, this rate (52.2%) was low compared with other studies. After 12 months, only 37.5% of patients maintained detectable antibody levels, while in other studies the comparable proportion were of 58% to 80%. In the occupational risk group a seroconversion rate of 70% was obtained, with 60% still positive after one year. This is also lower than in other studies as reported by DESMYTER et al. (1983), who showed a 100% seroconversion rate and maintenance of antibody in 98% after a period of one year<sup>9</sup>.

It is not clear whether these less satisfactory results can be attributed to differences of vaccine potency (batch to batch variation or degradation during storage or transport) or of host responses of the subjects under study.

One case of HBV infection occurred in a low responder patient 11 months after vaccination while circulating anti-HBs was still detected. The maximum antibody response was 9.1 SRU in the seventh month and had decreased to 2.5 SRU at the time of infection. At present, 7 months after infection, this patient remains HBsAg positive in a chronic carrier state. Among the occupational risk group, one case of clinical hepatitis occurred in a non-responder, with HBsAg appearing 8 months after the first dose of vaccine and clinical disease 1 month later. The clearance of HBsAg was observed after 6 months of antigenemia but no anti-HBs response has been so far elicited up to 4 months after clearance.

The reinforced vaccination schedule applicable in hemodialysis patients has been demonstrated to induce a better immune response<sup>4</sup>. Indeed, the fourth dose given to patients not only increased the seroconversion rate but its

more noticeable effect was in the increase of antibody levels (Fig. 3).

It has been reported in some efficacy trials that only those persons who respond well, with at least one serum sample presenting more than 10 SRU, are protected from HBV infection<sup>10, 14</sup>. Considering this criterion, in our study only 32.2% of patients and 60.0% of high risk personnel developed protective anti-HBs levels, which decreased to 13.5% and 25.0% respectively after twelve months.

In a trial conducted with Pasteur vaccine in a French hemodialysis unit, 5 patients who were low or non-responders during a period of one year, developed considerable anti-HBs levels after a booster injection<sup>1</sup>. From our results, it would appear desirable to administer a booster dose not only in patients but also in healthy persons to elicit a more persistent antibody level. Alternatively, for immunosuppressed individuals, HBV vaccine with higher HBsAg concentration (CLB — 27  $\mu$ g, Pasteur-10  $\mu$ g, MSD-40  $\mu$ g) have been employed with a significant improvement in protection level<sup>11, 12</sup>. Better results might be obtained with the use of these more immunogenic vaccines and we suggest their evaluation in our country.

## RESUMO

### **Resposta de anticorpo contra vacina de hepatite B inativada pelo calor (CLB-3 $\mu$ g) em pacientes de hemodiálise e pessoal de risco: Acompanhamento de um ano**

A resposta imune contra vacina de hepatite B (CLB-3 $\mu$ g) foi avaliada em 59 pacientes de hemodiálise e 20 funcionários de risco em adquirir infecção. A soroconversão foi observada em 52,5% e 70,0%, respectivamente. Um ano após a primeira dose, os níveis de anticorpos anti-HBs foram determinados em 37,5% dos pacientes e 60,0% dos funcionários. Os níveis de anticorpos foram expressos em unidades de radioimunoensaio (SRU = contagem da amostra sobre o controle negativo). Considerando apenas os indivíduos que responderam à vacina, no grupo de pacientes, 38,7% tiveram resposta baixa de anticorpos (2,1 — 9,9 SRU), 32,3% resposta média e 29,0% uma resposta elevada (> SRU), enquan-

to que no pessoal de risco, os valores foram 14,3%, 64,3% e 21,4%, respectivamente. Os autores sugerem o uso de vacinas de HBV com concentrações de HBsAg mais elevada ou um reforço no esquema de imunização para melhorar a resposta de anti-HBs, não só para pacientes como também para pessoas saudáveis.

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