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## Research Article

# Hemodialysis with Polymethylmethacrylate Restores the Response to Hepatitis B Vaccination in Chronic Dialysis Patients: Hypothesized Mechanism of Action

### Abstract

Patients undergoing hemodialysis often present with a reduced response to anti-hepatitis B virus (anti-HBV) vaccination. The soluble form of CD40 (sCD40) is elevated in hemodialysis patients and this has been shown to correlate with lack of response to anti-HBV vaccination. Due to its high molecular weight, conventional dialyzers cannot clear sCD40. Previous studies have demonstrated, that dialysis membranes in polymethylmethacrylate (PMMA) can reduce the levels of sCD40. We have studied the effect of dialysis with PMMA membranes in patients who were non-responders to anti-HBV vaccination after a complete cycle of vaccinations. Interestingly, we found that significantly more patients in the PMMA group were able to mount a response to vaccination, compared to the control group (P = 0.04).

## Materials and Methods

### Patients

Patients were included in this study if they had been on maintenance thrice-weekly dialysis treatment and had undergone at least one complete cycle of anti-hepatitis B virus vaccination (20 mg Fendrix, administered at 0, 1, 2 and 6 months) and were non-responders (e.g. anti-HBV antibody titre <10 UI/L). Patients were excluded from this study if they had undergone dialysis treatment with PMMA dialyzers prior to enrolment and/or if they had active neoplasia. All patients gave their informed consent to participate in the study. Following enrolment, patients were randomized into two groups, a control group that continued on the same dialysis treatment as previously, and a treatment group that was shifted to dialysis with PMMA series BK-F (1.3 to 2.1 m<sup>2</sup>). The treatment with PMMA continued until after the administration of the fourth vaccination. Patients underwent thrice-weekly dialysis for three months and were then administered the fourth vaccination dose. Immune response was evaluated hereafter.

### Laboratory parameters

Serum levels of sCD40 were measured every four weeks by enzyme-linked immunosorbent assay (ELISA) during the 12 weeks of study and again 4 weeks after anti-hepatitis B virus vaccination. ELISA was performed essentially as described by Contin et al. [2], all samples were measured in duplicates and the mean concentration was calculated. Blood urea nitrogen (BUN) concentrations, Kt/V and C-reactive protein (CRP) were evaluated every four weeks.

### Statistical analysis

Data were analyzed using Excel. Quantitative data were

## Introduction

Up to 50% of patients with end-stage renal disease (ESRD) undergoing dialysis therapy, present with a reduced response to anti-hepatitis B virus (anti-HBV) vaccination [1]. Patients on hemodialysis have been shown to have higher levels of soluble CD40 (sCD40), compared to healthy subjects [2,3]. Soluble CD40 is produced by B-cells by alternative splicing of the CD40 gene and by proteolytic cleavage of membrane-bound CD40 [4]. It has been shown experimentally that sCD40 reduces immunoglobulin (Ig) production and T cell activation and this molecule has therefore been hypothesized to have an immunodepressant effect [5]. Soluble CD40 is a glycoprotein and exists as dimeric and higher oligomeric forms of 50 and 150 kDa [6]. Due to its high molecular weight, sCD40 cannot be removed effectively by standard diffusive or convective dialysis therapy. Dialysis membranes in polymethylmethacrylate (PMMA) have been shown to effectively reduce the levels of sCD40 [7]. Reduction of sCD40 levels by hemodialysis was shown to correlate with an improvement in the response rate to anti-HBV vaccination [7]. We have shown previously, that dialysis with PMMA strengthens the response to anti-HBV vaccination and that this lasted effectively over time, even after discontinuation of PMMA [8].

The scope of the present study was to study the effect of dialysis treatment with PMMA on serum levels of sCD40 and the consequent response to anti-HBV vaccination in chronic hemodialysis patients who were non-responders to anti-HBV vaccination after a complete cycle of vaccinations.

presented in the form of mean and standard deviation. The Student *t*-test was used to compare data for the study group and the control group. Chi Square test was used to assess the independence of observed and expected data between the two groups. P was considered significant when <0.05.

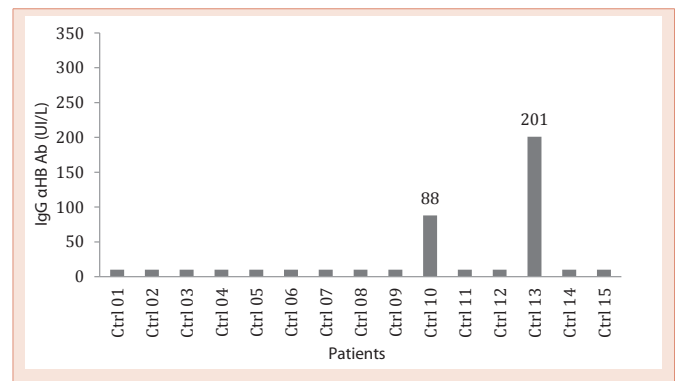
### Results

Thirty-two patients fulfilled the inclusion criteria and were enrolled in the study (average age 73±12 years). Following randomization 17 patients underwent dialysis with PMMA filters, and 15 patients underwent dialysis with either polysulfone or polyamide filters (Table 1). Significantly more patients in the PMMA group were able to mount a response to vaccination, compared to the control group ( $\chi^2$  test,  $p = 0.04$ ). Of the 17 patients who were dialyzed with PMMA filters, eight patients (47%) were able to develop a protective immune response against HBV (anti-HBs antibody levels > 10UI/L), whereas only two patients out of 15 in the control group (13%) developed a protective response (Table 2). In the PMMA group six out of the eight responders had anti-HBs antibody levels  $\geq 100$ UI/L (Figure 1), whereas only one of the responders in the control group had anti-HBs antibody levels  $\geq 100$ UI/L (Figure 2). This is relevant, because anti-HBs antibody levels  $\geq 100$ UI/L provide a very good seroprotection against HBV.

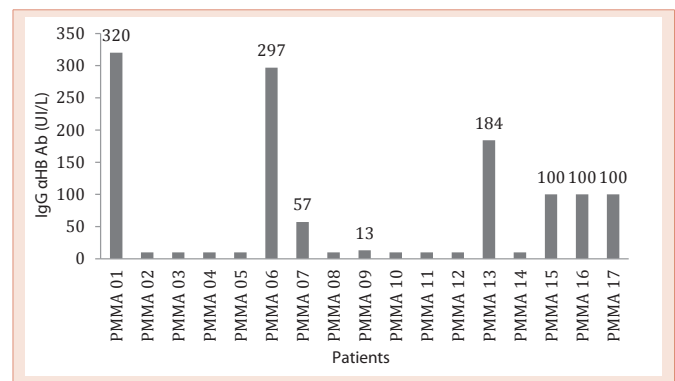
There were no significant differences in the level of sCD40 in the PMMA group and the control group throughout the study period (Figure 3). However, when dividing the data for the patients undergoing dialysis with PMMA membranes, into responders and non-responders, there was a noticeable (although not significant) decrease in sCD40 levels among the responders after the second month of dialysis, which was then followed by an increase back to baseline levels after the third month (Figure 4).

### Discussion

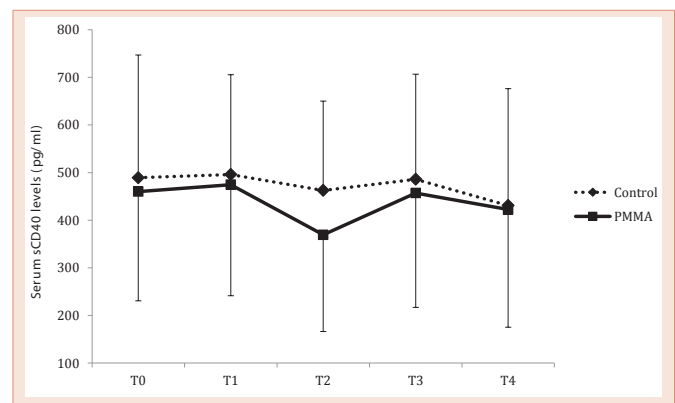
In the results presented here, we have shown that significantly more patients in the PMMA group were able to mount a response to vaccination, compared to the control group ( $\chi^2$  test,  $p = 0.04$ ). Of the



**Figure 1:** Anti-HBs antibody levels in control group. Anti-HBs antibody levels (UI/L) were measured one month after the last vaccine injection.



**Figure 2:** Anti-HBs antibody levels in PMMA (BK-F) group. Anti-HBs antibody levels (UI/L) were measured one month after the last vaccine injection.



**Figure 3:** Level of sCD40 in the PMMA group and the control group throughout the study period.

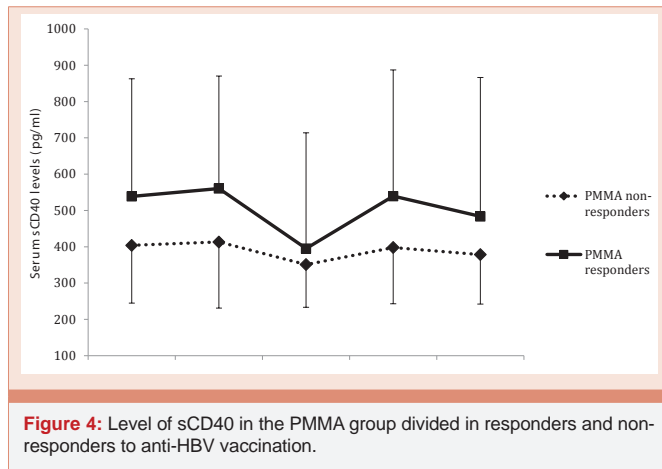
**Table 1:** Characteristics of patients who were randomized following one complete cycle of anti-HBV vaccination.

	Total	Controls	PMMA
Patients	32	15	17
Average age	73 ± 12	78 ± 9	67 ± 15
Gender (M/F)	18/14	8/7	10/7
Dry weight (kg)	74 ± 21	75 ± 17	73 ± 26
Dialysis vintage (months)	75 ± 58	97 ± 67	54 ± 48
Type of dialysis	Bicarbonate	Bicarbonate	Bicarbonate
Membrane	Polysulfone-polyamide	Polysulfone-polyamide	PMMA series BK-F
HBsAb (UI/L)	<10	<10	<10

**Table 2:** Results after the final dose of vaccination.

	Controls	PMMA	X2 test
Patients	15	17	
Patients with HBsAb > 10	2	8	
Percentage of responders	13 %	47 %	0.04

17 patients who were dialyzed with PMMA membranes, eight patients (47%) were able to develop a protective immune response against HBV (anti-HBs antibody levels > 10UI/L), whereas only two patients out of 15 in the control group (13%) developed a protective response. In the PMMA group six out of the eight responders had anti-HBs antibody levels  $\geq 100$ UI/L, whereas only one of the responders in the



control group had anti-HBs antibody levels  $\geq 100$ UI/L. Anti-HBs antibody levels  $\geq 100$ UI/L provide a very good protection against HBV. These results are in agreement with the results published previously, which demonstrated that 60% of patients undergoing dialysis with PMMA membranes mounted an immune response after three months of dialysis [7,8].

The sCD40 level was found to vary considerably from patient to patient. In the small patient population presented here, we found no significant difference between the PMMA group and the control group. After the second month of dialysis we observed a tendency towards a decrease in the PMMA group, although this was not significant. When the data from patients undergoing dialysis with PMMA membranes were divided into responders and non-responders, the decrease in serum sCD40 levels after the second month was even more noticeable in the responder group.

Patients with ESRD, undergoing hemodialysis, present with an alteration in immune response to anti-HBV vaccination [1]. Immunodeficiency in ESRD patients is caused by a dysregulation of various types of immune cells. The receptor CD40, which is expressed on the surface of B-cells, interacts with CD40 ligand (CD40L), which is expressed on T cells, NK cells and basophils [9]. The CD40/CD40L complex modulates the proliferation of B-cells, expression of IL-12 and activation of T-cells [9]. The soluble form of CD40, sCD40, serves to regulate the interaction between CD40 and CD40L and thereby causing a reduction in lymphocyte activation and Ig production. A possible explanation to the noticed drop in sCD40 levels after the second month of dialysis could be its removal by the PMMA membrane. This drop would be enough to generate Ig and thereby to mount a protective response against HBV. However, the removal of sCD40 would also cause an increase in lymphocyte activation by CD40/CD40L, leading to proliferation of B-cells and thereby an increase in sCD40 expression, thus explaining the subsequent increase in sCD40 levels after 3 months of dialysis. Further studies are required to fully elucidate the mechanism of sCD40 regulation.

In our precedent work [8] we underlined that PMMA is the only membrane able to absorb medium and large molecules, including

sCD40 and high-molecular weight protein bound uremic toxins (PBUTs) involved in other major comorbidities such as uremic pruritus, anemia, and amyloidosis ecc. In addition to altered immune-response.

Therefore, following this line of thought super-flux dialyzers with high convection and adsorption such as the PMMA-BK series could be widely used in clinical situations where large quantities of High Molecular Weight Toxins (HMWT) or PBUTs are produced including septic patients with acute renal failure in which it is necessary to control at the same time uremia, fluid status and the removal of cytokines.

We wondered whether there could be a rationale for an occasional use of PMMA in order to maintain better immune competence during and after every vaccination and also strengthen the immune system of dialyzed patients.

So, in conclusion, PMMA membranes should become the first choice treatment for immune depressed patients, as well as for patients awaiting kidney transplantation in whom a good response to anti-HBV is recommended. Looking at recent findings sCD40 removal could play, in the near future, a role in HD patients improving endothelial function and preventing some dialysis comorbidities (i.e. artery coronary disease) [10].

In addition we should resolve the important doubt whether continuous or periodic use of PMMA (i.e. one or two sessions per month) could improve immune dysfunction in all dialysis patients who seem to be particularly immune compromised. Further studies are needed to clarify the possible role of periodical – intermittent dialysis sessions with PMMA adsorptive membranes in the removal of HMWT that are produced daily.

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