A 5-Year Follow-Up on Antibody Response After Diphtheria and Tetanus Vaccination in Hemodialysis Patients

Sabine Krüger, MD, Michael Müller-Steinhardt, MD, Holger Kirchner, MD, and Burkhard Kreft, MD

Chronic renal failure is associated with a T-cell–dependent immune defect. In the past, various studies have focused on the insufficient immune response to vaccination of hemodialysis patients. An impaired vaccination response rate has been reported for vaccines against hepatitis B, influenza, tetanus, diphtheria, and others. However, no data exist on the long-term success of vaccination against tetanus and diphtheria in these patients. The aim of the present study is to investigate seroresponse to tetanus and diphtheria vaccination over a 5-year period. Antibody levels were determined by enzyme immunoassay. Antidiphtheria antibody levels of 31 hemodialysis patients were determined 5 years after vaccination. After 5 years, 10 of 31 patients (32%) had a protective antibody level against diphtheria (>0.1 IU/mL), whereas 12 months after vaccination, 26 of 71 patients (37%) were protected. Also, mean antibody levels significantly decreased. Furthermore, antitetanus antibody levels of 21 patients simultaneously vaccinated against tetanus and diphtheria were investigated. After 5 years, 15 of 21 patients (71%) were protected compared with 46 of 71 patients (65%) in the hemodialysis collective studied after 12 months. In the interval between 1 and 5 years after vaccination, significantly more patients in the initial nonresponder group had died than in the responder group; therefore, the overall protection rate observed in vaccinated patients increased. Our results provide evidence that during a 5-year period, antibody persistence against tetanus toxoid is better than that against diphtheria toxoid. Therefore, detection of individual antibody concentrations may be indicated to decide on revaccination.

© 2001 by the National Kidney Foundation, Inc.

INDEX WORDS: Diphtheria vaccination; tetanus vaccination; long-term response; hemodialysis (HD); immune defect.

Patients with chronic renal failure have been shown to be more susceptible to infections with viruses, bacteria, and fungi than healthy individuals.1 The immunodeficiency is associated with decreased immunoglobulin production, diminished interleukin-2 secretion by T lymphocytes, and impaired macrophage functions (reviewed in2,3). At present, one cause of multiple immunologic disorders is believed to be a defect in costimulatory properties of antigen-presenting cells.4 Several studies have focused on the protection of these patients by vaccination. These investigations have shown that compared with healthy controls, the response to vaccination with various bacterial and viral vaccines is impaired in hemodialysis patients. To date, diminished seroconversion rates have been reported for vaccination against hepatitis B,5 tetanus,6 diphtheria,7 pneumococcal infections,8 and influenza.9

In an earlier study by our group, the response to tetanus and diphtheria vaccination was investigated. In 228 hemodialysis patients with an unknown vaccination history or no documented diphtheria vaccination, a protection rate against diphtheria of 22% was found.7 After immunization of 71 patients, only 26 patients (37%) seroconverted. Seventy-one of the 228 hemodialysis patients with an unknown tetanus vaccination history had been tested additionally for antitetanus toxoid antibody levels.6 Only 31 patients (44%) had sufficient protection against tetanus compared with 70% of healthy individuals,10 and 15 patients (38%) seroconverted after vaccination. We found a correlation between impaired immune response to tetanus and diphtheria toxoid, which are both T-cell–dependent vaccines.6 However, no long-term results on the response to vaccination against tetanus and diphtheria have been reported.

For this reason, we studied the outcome of 176 of the 228 hemodialysis patients included on our study 5 years ago. Thirty-five of these patients had undergone renal transplantation within the
last 5 years. We hypothesize that diphtheria and tetanus protection rates further decreased in hemodialysis patients and normal renal function after transplantation contributed to the maintenance of antibody levels.

METHODS

Patients and Serum Sampling

Two hundred twenty-eight hemodialysis patients with an unknown vaccination history were tested for protection against diphtheria 5 years ago. Clinical characteristics of the patients have been described previously.\(^6,7\) For the present study, 176 of these patients (101 men, 75 women) from three of the initial four dialysis centers were followed up. Patients from the fourth center had not been vaccinated. Initially, a blood sample was obtained for the detection of antibody levels for diphtheria and tetanus, and those patients who did not have vaccination documents or had not been immunized before were inoculated at the same time \((n = 95).7\) Seventy-one of these patients were simultaneously vaccinated with tetanus and diphtheria toxoid at 0 months (40 international units [IU] tetanus toxoid + 4 IU diphtheria toxoid; charge 251021 A; Chiron-Behring, Marburg, Germany). Some of these patients were subsequently immunized with diphtheria toxoid (4 IU/injection; charge 26071 and 27011; Chiron-Behring) at 1 and 6 months. No revaccination had been performed within the previous 5 years. For evaluation of the long-term response, all initially vaccinated patients from whom blood was available after 5 years were included. Antidiphtheria toxoid antibody levels were determined in 31 hemodialysis patients and 15 transplant recipients.

For investigation of the protection rate against tetanus, the 71 initially vaccinated hemodialysis patients were followed up. For the present study, antitetanus toxoid antibody titers were investigated in 21 patients still on hemodialysis therapy and 10 of 15 renal transplant recipients. Blood samples were drawn before hemodialysis or at transplantation aftercare examinations; serum was fractionated and stored at \(-80^\circ C\) until antibody detection.

Detection of Diphtheria and Tetanus Antitoxoid Antibodies

To determine antibody levels against diphtheria toxoid, an enzyme immunoassay was used (Immuno, Heidelberg, Germany). Antibody levels were determined in duplicate by incubation of serum samples on diphtheria toxoid–coated microtiter plates. Peroxidase-bound diphtheria toxoid was used for quantification. The antitoxin standard was calibrated by the current World Health Organization standard method involving intracutaneous injection into guinea pigs (Schick test, expressed in international units per milliliter). The lower limit of detection was 0.01 IU/mL. Antibody concentrations obtained were classified according to the manufacturer’s recommendations as inadequate protection \(<0.1\) IU/mL) and protection \(\geq 0.1\) IU/mL). Effective seroconversion, defined in the previous study, was an antibody concentration of 0.1 IU/mL or greater 3 or 6 months after vaccination.

Concentrations of antibodies against tetanus toxoid also were determined by enzyme immunoassay (Immuno) using tetanus toxoid–coated microtiter plates. The enzymatic turnover of tetramethylbenzidine by tetanus toxoid–linked peroxidase was measured at 450 nm. Protection against tetanus was defined as an antibody level greater than 0.1 IU/mL, and seroconversion, as an increase of antibody levels from less than 0.1 IU/mL to 0.1 IU/mL or greater. These methods were identical to those used for the detection of diphtheria and tetanus toxoid antibody levels in our previous studies.\(^6,7\)

Statistical Analysis

Antibody concentrations were determined in duplicate, and the mean was used for statistical analysis. Comparison of two independent groups was performed using the Mann-Whitney \(U\) test. Categories were compared by Fisher’s exact test. Protection status after simultaneous tetanus and diphtheria vaccination was compared by McNemar test. \(P\) less than 0.05 is considered significant.

RESULTS

Long-Term Immunity Against Diphtheria in Hemodialysis Patients

Within the past 4 years, 76 of the 176 patients followed up had died, 6 patients had moved, 35 patients had undergone renal transplantation, 49 patients remained on hemodialysis therapy, and the history of 10 patients was unknown. Antibody levels were determined in 31 patients still on hemodialysis therapy and 15 renal transplant recipients who had been initially vaccinated. The 18 other HD patients and 12 of the transplant recipients had not been vaccinated. Of the remaining 8 transplant recipients, no serum was available because they were no longer in contact with their former hemodialysis center and did not reply to a personal letter. At the time of this study, 10 of these 31 hemodialysis patients (32%) had protection against diphtheria, whereas 1 year after vaccination, 13 of the same 31 patients (42%) were protected (Fig 1). In patients with no initial protection and subsequent vaccination, antitoxoid levels decreased significantly \((P < 0.05, \text{ Mann-Whitney } U \text{ test})\) from 0.26 ± 0.48 IU/mL 1 year after vaccination to 0.12 ± 0.26 IU/mL after 5 years (Fig 2).

The rate of protection in renal transplant recipients also decreased from 52% \((n = 23)\) after 1 year to 40% \((n = 15; \text{ Fig 1})\). Considering only those 15 patients studied after 5 years, the protection rate after 1 year had been even greater (67%). However, differences in protection rates between renal transplant recipients, dialysis pa-
patients, or patients who died were not significant. Antibody levels in transplant recipients without initial protection and subsequent vaccination \((n = 12; 0.06 \pm 0.03 \text{ IU/mL before vaccination})\) significantly increased 1 year after vaccination \((0.33 \pm 0.52 \text{ IU/mL})\) and significantly decreased in the interval 1 to 5 years after vaccination \((0.14 \pm 0.23 \text{ IU/mL}; P < 0.05, \text{ Mann-Whitney } U \text{ test})\). No correlation between sex and vaccination response was found.

**Long-Term Immunity Against Tetanus in Hemodialysis Patients**

For investigation of long-term immunity against tetanus, all of the 71 vaccinated hemodialysis patients were followed up. Twenty-eight patients had died, 3 patients had moved, 15 patients had undergone renal transplantation, the history of 4 patients was unknown, and 21 patients are still on hemodialysis therapy. For the present study, antibody levels of all 21 dialysis patients and 15 transplant recipients could be determined. Five years after vaccination, 15 of 21 vaccinated hemodialysis patients \((71\%)\) had protective antibody levels \((\geq 0.1 \text{ IU/mL}; \text{ Fig 3}), \) whereas 1 year after vaccination, 16 of these 21 patients \((76\%)\) had been protected.

In our previous study, we showed that the protection rate in the 71 patients after immunization was 65\%. These differences were not statistically significant; however, we questioned whether the reason underlying this seeming increase in protection rate was greater mortality in the nonresponder group. Statistical analysis showed that of those patients who had died within the previous 4 years, significantly \((P < 0.019; \text{ Fisher's exact test})\) more persons \((54\%)\) did not respond to tetanus vaccination than in the hemodialysis group \((24\%)\). In those patients without initial protection and subsequent vaccination,
antibody levels increased significantly 1 year after inoculation \((P < 0.05, \text{Mann-Whitney } U\text{ test})\); Fig 4). Geometric mean level increased from 0.21 ± 0.34 IU/mL after 1 year to 0.49 ± 0.83 IU/mL after 5 years postimmunization; however, this increase was not significant.

Consequently, we questioned whether transplant recipients, with naturally less concomitant diseases, had shown a better response to vaccination. Concerning protection 1 year after vaccina-

tion, the rate of immunity against tetanus was significantly \((P < 0.05, \text{Fisher's exact test})\) greater in patients with subsequent renal transplantation (80%) than in the group of patients who died within the next 4 years (46%; Fig 3). In transplant recipients without protection before vaccination \((n = 4)\), the increase in mean antibody level from before \((0.04 ± 0.01 \text{ IU/mL})\) to 1 \((0.68 ± 1.25 \text{ IU/mL})\) and 5 years \((0.78 ± 1.48 \text{ IU/mL})\) after vaccination was because of the small patient number, which was not significant, but showed the same trend as in hemodialysis patients.

In contrast to our previous study, there was no difference between men and women in long-term protection against tetanus.

**Comparison of Long-Term Protection Against Diphtheria and Tetanus**

Concerning antibody maintenance over 5 years, 21 hemodialysis patients simultaneously immunized against diphtheria and tetanus were tested for protection. The long-term response against tetanus clearly differed from the immune response to diphtheria toxoid \((P = 0.039, \text{McNemar test})\). Fifteen of 21 patients (71%) had protective antibody levels for tetanus, whereas 7
of these 21 patients (33%) were protected against diphtheria (Table 1).

**DISCUSSION**

The immune response to various vaccines is impaired in hemodialysis patients. Most studies have focused on immunization against hepatitis B because of the increased risk for infection or infecting others as a result of the dialysis procedure. Different investigators found decreased response rates in hemodialysis patients, varying between 57% and 89%11-13 compared with greater than 90% in healthy controls.14 Furthermore, vaccination against *Streptococcus pneumoniae* and influenza virus9 resulted in diminished antibody production in patients with chronic renal failure. In earlier studies, we focused on immunity against tetanus and diphtheria, two diseases with decreasing immunity in the western population because of insufficient vaccination.10,15

Historically, antitoxin levels for diphtheria and tetanus were determined by serum toxin neutralization tests in animals,16,17 which are not suitable for the screening of large numbers of subjects. Therefore, other in vitro test systems have been established, eg, hemagglutination, neutralization in cell-culture systems, and enzyme-linked immunosorbent assays, which have been widely used for prevalence and vaccination response studies.18,19 However, because of the low incidence of tetanus and diphtheria, no studies of protective antibody concentration could be performed. Existing definitions of protection and seroconversion were taken from case reports and animal studies. Values used in our previous and present studies follow the guidelines of the manufacturer, as well as World Health Organization recommendations.18,19 The commercial enzyme-linked immunosorbent assay used in our study has been used before for the investigation of antitetanus and antidiphtheria antibodies in a healthy northern German population.20,21

In our hemodialysis collective, we showed that 1 year after vaccination, impaired response to diphtheria toxoid correlated with impaired response to tetanus toxoid.6,7 However, no long-term data (>3 years) on vaccination response neither to tetanus and diphtheria toxoid nor to other vaccines are available to date for hemodialysis patients.

Despite multiple immunization programs, recent studies indicate that healthy individuals in western Europe showed poor protection rates against diphtheria (31% with antitoxin levels ≥0.1 U/mL).22 In our hemodialysis collective 1 year after immunization, 37% of patients (n = 71) were protected against diphtheria. Five years after vaccination, the protection had further decreased from 37% to 32%. Protection after 1 year in the 31 patients still on hemodialysis therapy after 5 years was even greater (42%). Patients with protection at the time of inoculation also were included in this group of patients. They provided an indication of vaccination because of unknown vaccination history and represent a normal dialysis collective. For evaluation of antibody maintenance, only those patients without protection before vaccination were included. Within the last 4 years, the antibody level decreased significantly in hemodialysis patients.

Against the background of the recent epidemic of diphtheria in the former Soviet Union, which peaked in 1994 and 1995 with 50,000 annual reported cases,23,24 the decreasing prevalence of antitoxin antibodies in the western population20-22 and insufficient vaccination response of hemodialysis patients, together with reduced long-term antibody maintenance, the establishment of appropriate revaccination schedules is of prime importance. Nicolay et al25 previously showed that in healthy individuals, an additional booster injection failed to increase protection rates significantly. However, this needs to be investigated further in chronic renal failure. We questioned whether restoration of renal function by transplantation contributed to the maintenance of protection. In the 15 vaccinated patients who underwent transplantation in the period 1 to

<table>
<thead>
<tr>
<th>Table 1. Comparison of Protection Status 5 Years After Vaccination in 21 Hemodialysis Patients Simultaneously Vaccinated Against Tetanus and Diphtheria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetanus</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Diphtheria</td>
</tr>
<tr>
<td>Protected</td>
</tr>
<tr>
<td>Unprotected</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

NOTE. *P* < 0.05, McNemar test.
5 years after vaccination, protection decreased from 67% after 1 year to 40% after 5 years. This decrease also was seen in mean antibody levels and might be reinforced by immunosuppressive medication after transplantation.

For vaccination against tetanus, we obtained different long-term results. In patients still on hemodialysis therapy 5 years after vaccination, we found greater protection rates than in the collective studied after 1 year (71% versus 65%). However, considering immunization rates 1 year after vaccination only in those patients still on hemodialysis therapy after 5 years, the protection rate decreased slightly (76% after 1 year versus 71% after 5 years). Possibly because of low patient numbers available after 5 years, these differences were not statistically significant, but still required an explanation.

We compared protection rates 1 year after vaccination in the groups of patients who died, transplant recipients, and those still on hemodialysis therapy and found that among patients who died of secondary reasons, significantly more were nonresponders than those still alive. Therefore, our results provide evidence for greater mortality among nonresponders to the tetanus vaccine. Similar to diphtheria protection, renal transplantation had no beneficial effect on specific antibody maintenance. The association between the response to diphtheria and tetanus vaccination shown in our previous study was not confirmed after 5 years. After 1 year, patients who did not respond to diphtheria toxoid also failed to develop protective antibody levels against tetanus. Instead, the protection rate after 5 years was significantly greater against tetanus than diphtheria.

On the basis of results discussed here, we conclude that short-term results of vaccination protocols against tetanus and diphtheria in hemodialysis patients cannot represent the immune status of those who are still on hemodialysis therapy 5 years later. Although our patients lost some protection against diphtheria, they showed a far better ability to maintain protection against tetanus. Therefore, we recommend antibody monitoring and sufficient boosters for diphtheria. To date, no appropriate revaccination protocols have been established for hemodialysis patients. This needs to be studied in the future. Special attention should be given to nonresponders against tetanus vaccine, and further studies on the predictive value of nonresponse to immunization for the survival of hemodialysis patients would be of great interest.

REFERENCES

15. De Melker HE, Berbers GAM, Nagelkerke NJD,