Trivalent influenza vaccine in patients on haemodialysis: impaired seroresponse with differences for A-H3N2 and A-H1N1 vaccine components

W.E.P. Beyer*, D.J. Versluis†, P. Kramer‡, Ph.P.M.N. Diderich, W. Weimar† and N. Masurel***

One hundred and one patients on haemodialysis, 21 patients on peritoneal dialysis and 30 healthy controls received a trivalent split vaccine containing 15 μg haemagglutinin of a recent influenza A-H3N2, influenza A-H1N1 and influenza B strain, respectively. Antibody production after four weeks was determined by the haemagglutination-inhibition test and expressed as response rate, protection rate and overall mean fold increase. The patients on haemodialysis revealed a diminished seroresponse, as compared to patients on peritoneal dialysis and controls. For influenza A-H3N2, this was less distinct than for the other two antigens. In patients on haemodialysis the protection rate was 66% against the A-H3N2 vaccine component (versus 85% in controls, not significant), but only 25% against A-H1N1 and 27% against B (versus 84 and 77% in controls, $p < 0.001$). Duration of haemodialysis up to eight years did not affect seroresponse. Patients on haemodialysis who were primed for influenza A-H1N1 in the period 1947–1957, reacted markedly better to the A-H1N1 vaccine component than subjects of other priming periods. A booster injection of the same vaccine dosage four weeks after the first immunization, performed in 98 patients on haemodialysis, was of little value: it had virtually no effect with regard to influenza A-H1N1 and influenza B, and showed, though significantly better, still poor results for A-H3N2. The differences in seroresponse between the A-H3N2 and A-H1N1 vaccine component suggest a major defect of primary, and a minor defect of secondary humoral response in patients on haemodialysis. The consequences for vaccine policy in these patients are discussed.

Keywords: Influenza vaccine; haemodialysis; peritoneal dialysis; immunogenicity

Introduction

Patients with chronic renal disorders may benefit from annual vaccination against influenza A and B viruses as excess mortality due to epidemic influenza has been reported in this group[1]. Protection provided by inactivated whole virus, split or subunit influenza vaccines is mainly associated with the evocation of high antibody titres against viral haemagglutinin (HA)[2]; the role of simultaneously stimulated cellular immunity is less clear. Patients with chronic renal failure have an impaired cellular and humoral immunity (for review see [3]) which might also affect seroresponsiveness to vaccines. The immunogenicity of influenza vaccine has been studied in patients with glomerular diseases[4,5] and patients on haemodialysis[5–8], and also in kidney transplant recipients[7,9–13]. While the safety of the vaccine in these patient groups was established, varying results were obtained in reaching a satisfactory humoral response. However, in most studies small numbers of patients were involved. Recently, an evidently impaired seroresponse to influenza vaccine has been described in 40[14] and 29[15] patients on haemodialysis. In the present study, the anti-HA-antibody production of trivalent influenza vaccine in a large number of patients on haemodialysis has been evaluated. The influence of the patients' age and the duration of the dialysis treatment on antibody response was studied.

Materials and methods

Study population

One hundred and sixty five subjects were included in the study. The group of patients on intermittent maintenance haemodialysis (HD) consisted of 108 patients (53 males and 55 females), aged 17 to 76 years. In 18 patients (17%), the underlying disorder leading to endstage renal failure was of immunological origin.

*Department of Virology and WHO Influenza Centre and †Department of Internal Medicine I, Erasmus University Rotterdam/ University Hospital Dijkzigt, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands. ‡Department of Internal Medicine, St. Clara Hospital, Rotterdam, The Netherlands. **Department of Internal Medicine, St. Franciscus Gasthuis, Rotterdam, The Netherlands.
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Haemodialysis had started 1 month to 13 years before. For comparison, 26 patients undergoing continuous ambulatory peritoneal dialysis (PD) were studied. This group consisted of 14 males and 12 females, aged 23 to 73 years. An underlying immunological disorder had caused renal failure in 9 patients (35%). Dialysis had started 2 months to 12 years before. None of the HD or PD patients received immunosuppressive drugs at the time of vaccination. All patients were in a stable condition. 31 healthy volunteers (15 males, 16 females, age range 19–69 years) served as controls for the immunogenicity of the vaccine.

Vaccine, dosage, administration, vaccination regimen

0.5 ml of a trivalent influenza split vaccine for the season 1985–1986 (Alorbat, Asta-Werke, Frankfurt/Main, FRG, lot no. 075104) containing 15 μg HA of A/Philippines/2/82 (H3N2), A/Chile/1/83 (H1N1) and B/USSR/100/83, respectively, was administered by intramuscular injection in the upper arm on day 0 (first immunization) and on day 30 (booster immunization). Sera were obtained on day 0 (S0, prevaccination), day 30 (S30) and day 60 (S60). The study was performed in The Netherlands in the absence of naturally occurring influenza in the autumn of 1985.

Laboratory investigations and calculations

Sera were separated immediately after blood collection and clotting and kept frozen at –20°C until titration. Influenza virus strains identical to the vaccine components (kindly provided by Dr J.J. Skehel, WHO World Influenza Centre, London, UK) were propagated in embryonated 10 to 12-day-old chicken eggs. Because of the low avidity of the influenza B virus, infectious egg fluids of this strain were treated with ether according to Berlin *et al.*[16] and the watery phase was used in the serologic tests.

Serum haemagglutination-inhibition (HI) titres were determined twice by standard methods[17] simultaneously in pre- and postvaccination sera. Titres were expressed as the reciprocals of the dilution showing 50% haemagglutination inhibition with 3 haemagglutination units of the antigen. From the results of the two determinations per serum and per antigen, the geometric means were used for further calculations. Negative titres (<9) were arbitrarily regarded as 5.

With the method used, protection against infection is associated with an HI-titre of 100 against influenza A[18,19] and 200 for ether-treated influenza B[20]. Separately for each antigen, subjects who had a prevaccination titre above these thresholds, were excluded. The results of the previously unprotected individuals were used for calculation of the following measures. (1) Response rate, i.e. the proportion of subjects who showed a fourfold or greater titre rise after vaccination. (2) Protection rate, i.e. the proportion of subjects exceeding the threshold titre of 100 (influenza A) and 200 (influenza B) respectively, after vaccination. (3) Overall mean fold increase (MFI), i.e. the difference between the logarithmic ($\log_{10}$) geometric mean titres of post- and prevaccination sera. Differences in qualitative measures were tested for significance by the χ^2 test and in quantitative measures by the Wilcoxon rank test.

Results

Final groups and clinical reactions

Twenty one subjects did not complete the full course of the vaccination scheme because of performance of a renal transplantation (one HD patient and three PD patients), development of dialysis-associated peritonitis (six PD patients), death for reasons unrelated to the vaccination (three HD patients) or unknown reasons (six HD patients and two control subjects). One hundred and one HD patients, 21 PD patients and 30 control subjects completed the first vaccination (S0 + S30), and 98 HD patients, 17 HD patients and 29 control subjects the booster vaccination (S0 + S30 + S60). No major clinical side effects of the vaccine were reported.

Seroresponse to first vaccination

The height of prevaccination titres was inversely related to the height of titre rises after vaccination (not shown). After exclusion of subjects with already protective prevaccination titres for each antigen separately, the HD and PD groups and the control group were tested for differences with regard to geometric means of prevaccination titres, male/female ratio and age. No significant differences were found, and the groups were regarded as comparable.

As shown in *Table 1*, the control subjects produced satisfying protection and response rates ranging from 77 to 95%. Results of PD patients were inferior to controls, but no statistical difference could be detected, except for the MFI value for the A-H3N2 component (1.35 versus 1.79, $p = 0.02$) and the protection rate for the A-H1N1 component (47% versus 84%, $p = 0.01$). In contrast, the patients on haemodialysis showed a significant impairment for all three vaccine components: only 67, 43 and 56% of 101 HD patients showed

Table 1 Response rates, protection rates and mean fold increase (MFI) values in control subjects and patients on peritoneal dialysis (PD) and on haemodialysis (HD), after first vaccination

Parameter	A-H3N2			A-H1N1			B		
	Controls	PD	HD	Controls	PD	HD	Controls	PD	HD
Subjects included/ subjects vaccinated[a]	20/30	18/21	73/101	25/30	19/21	91/101	26/30	18/21	84/101
$\log_{10}$ GMT S0[b]	1.14	1.20	1.10	1.12	0.96	1.09	1.16	1.21	1.29
Response rate (number, %)	19(95%)	15(83%)	49(67%)*	21(84%)	9(47%)**	39(43%)***	21(86%)	14(78%)	47(56%)*
Protection rate (number, %)	17(85%)	17(94%)	48(66%)†	21(84%)	15(79%)	23(25%) ***†††	20(77%)	9(50%)	23(27%)***
MFI ± s.d.	1.79 ± 0.63	1.35 ± 0.63*	1.09 ± 0.73***	1.27 ± 0.54	1.02 ± 0.60	0.58 ± 0.51 ***††	1.46 ± 0.71	1.20 ± 0.65	0.75 ± 0.64 ***†

[a]Number of subjects after exclusion of those with protective prevaccination titres (≥ 100, influenza A; ≥ 200, influenza B)/total number of subjects vaccinated. [b]Logarithmic geometric mean titre of prevaccination sera. Significant differences between groups by χ^2 test (response rate, protection rate) or Wilcoxon-test (MFI): *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus controls. †$p < 0.05$, ††$p < 0.01$, †††$p < 0.001$ versus PD

Table 2 Complete nonresponders among control subjects and patients on peritoneal dialysis (PD) and on haemodialysis (HD), after first vaccination

Parameter	Control	PD	HD
Number of subjects unprotected against all three vaccine components prior to immunization/total number of subjects vaccinated	16/30	14/21	61/101
Complete nonresponders after first vaccination	–/16(0%)	–/14/(0%)	13/61(21%)*
Subjects remaining unprotected after first vaccination	1/16(6%)	1/14(7%)	15/61(25%)

*$p < 0.05$ versus controls

a fourfold or greater titre rise and only 66, 25 and 27% obtained protective titres against influenza A-H3N2, A-H1N1 and B, respectively. This impairment was less distinct for A-H3N2 than for A-H1N1 and B.

The significance of these findings is stressed by the calculation of complete nonresponders (*Table 2*). Sixty one HD patients were not protected against all three influenza strains prior to immunization. Of these, still 13 (21%) did not respond to any of the three vaccine components and 15 (25%) remained unprotected against all three viruses after the first vaccination. In contrast, no complete nonresponders could be found among 14 PD patients and 16 controls.

Variables influencing seroresponse in dialysis patients

The following variables were tested for influencing the responsiveness to the vaccine components in the various groups: duration of dialysis treatment, age, sex and etiology of renal disease (immunological or not). The latter two did not show any influence. *Figure 1* presents the seroresponse of HD patients, expressed as increases in individual titres related to the duration of haemodialysis in years. The distribution remained constant during the first 8 years. The few patients with a longer duration of dialysis more frequently produced higher titre increases, similar to healthy controls. Therefore, in *Table 3*, the serological response is summarized for two classes of HD patients (up to 8 years versus more than 8 years of haemodialysis). Again, there were no significant differences between these classes in respect of male/female ratio, age, prevaccination titres or etiology of renal failure. Indeed, the patients maintained on HD for more than 8 years achieved response and protection rates similar to control subjects, while the rates in the classes with shorter duration were lower. This phenomenon was present for all three antigens, but most distinct for A-H1N1.

A similar analysis for peritoneal dialysis was not appropriate as there was only one PD patient (after exclusion of subjects with high prevaccination titres) whose dialysis treatment had started more than 8 years ago. In the other subjects no correlation between

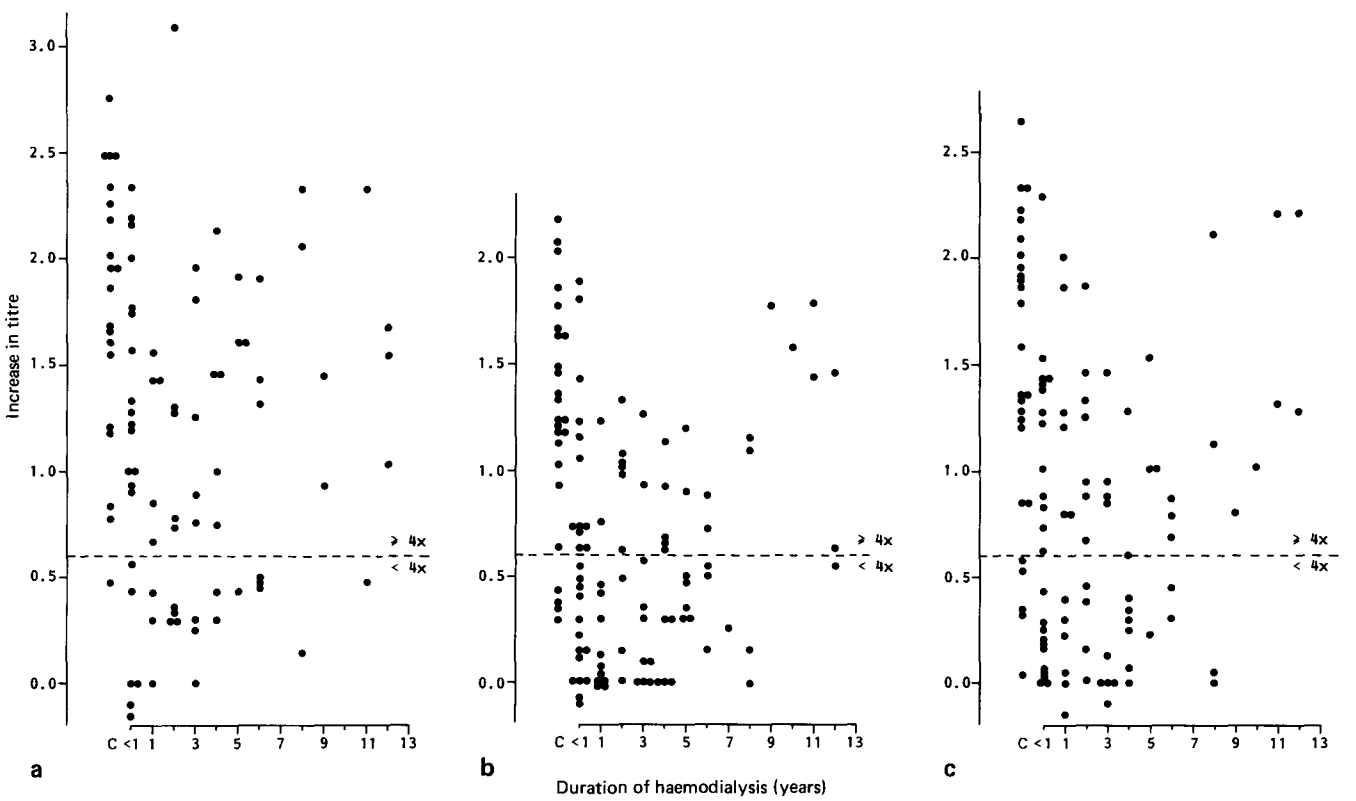

Figure 1 Individual increase in pre- and postvaccination titres among patients on haemodialysis after first vaccination, related to duration of haemodialysis. Increase in titre is expressed as log (S30) – log (S0). C, control subjects. ----, Threshold of nonresponders (increase less than fourfold) and responders (increase fourfold or greater). a, influenza A-H3N2; b, influenza A-H1N1; c, influenza B

Table 3 Response rates, protection rates, and mean fold increase (MFI) values in patients on haemodialysis (HD) after first vaccination. Differences between duration of haemodialysis ≤8 years and >8 years

Parameter	A-H3N2			A-H1N1			B		
	HD ≤ 8 years	HD > 8 years	*p* value	HD ≤ 8 years	HD > 8 years	*p* value	HD ≤ 8 years	HD > 8 years	*p* value
Subjects included[a]	66	7		84	7		78	6	
Log GMT S0[b]	1.09	1.29	n.s.	1.10	1.13	n.s.	1.32	0.88	n.s.
Response rate (number, %)	43(65%)	6(86%)	n.s.	33(39%)	6(86%)	<0.05	18(23%)	6(100%)	<0.001
Protection rate (number, %)	42(64%)	6(86%)	n.s.	17(20%)	6(86%)	<0.001	21(27%)	2(33%)	n.s.
MFI ± s.d.	1.06 ± 0.75	1.35 ± 0.60	n.s.	0.51 ± 0.49	1.32 ± 0.51	<0.01	0.70 ± 0.61	1.48 ± 0.59	<0.05

[a]Number of subjects after exclusion of those with protective prevaccination titres (≥ 100, influenza A; ≥ 200, influenza B). In total, 101 HD patients were vaccinated. [b]See *Table 1*. n.s., not significant

duration of peritoneal dialysis and the findings of seroresponse was found (not shown).

In the control and PD group, the seroresponse was not influenced by the age of the vaccinees. In HD patients, however, there was a tendency to a decreasing seroresponse with increasing age for all three antigens (*Figure 2*). The age distribution for A-H1N1 in HD patients differed markedly from the A-H3N2 and the B component in showing a normal seroresponse in subjects aged 36–45 years reaching response and protection rates of 86 and 86% and an MFI of 1.04 (versus 84, 84% and 1.27, respectively, for controls, not significant). This peak was not due to unequivocal distributions of prevaccination titres or other variables in the different age cohorts. All other ages responded significantly inferiorly, as is presented in *Table 4*. For the

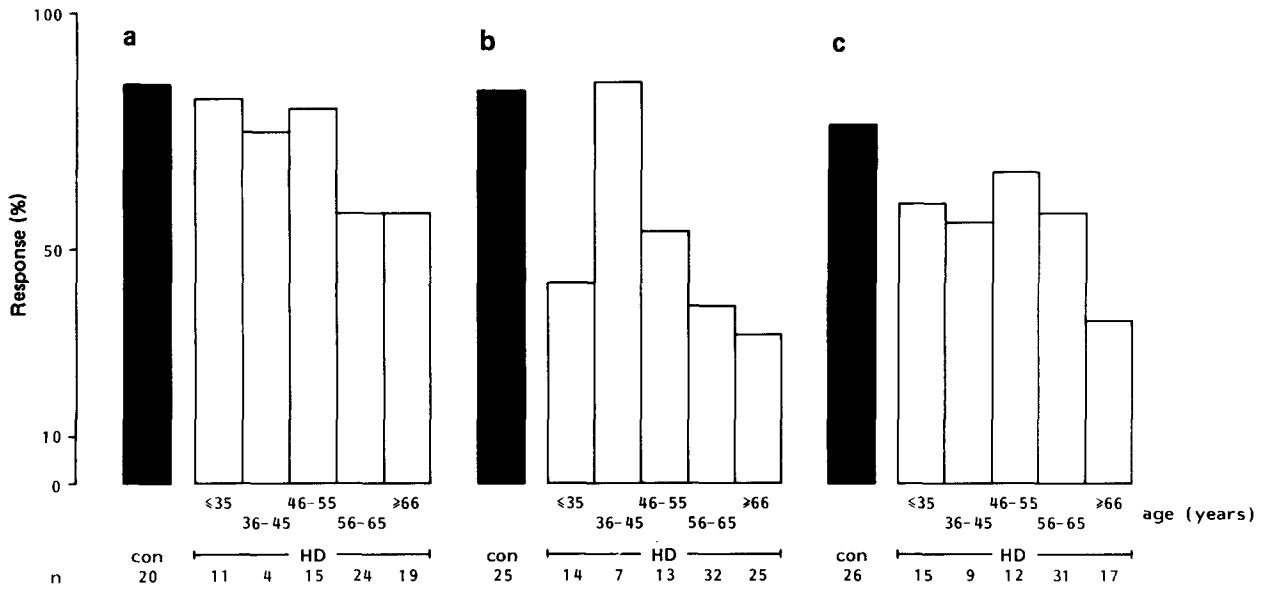

Figure 2 Response rates in different age cohorts of haemodialysis patients. a, Influenza A-H3N2; b, influenza H1N1; c, influenza b. ■, con, control subjects; □, HD, patients on haemodialysis; n, number of subjects

Table 4 Response rates, protection rates and mean fold increase (MFI) values in patients on haemodialysis after first vaccination. Differences between age cohorts 36–45 years and others

Parameters	A-H3N2			A-H1N1			B		
	36–45 years	Others[c]	*p* value	36–45 years	Others	*p* value	36–45 years	Others	*p* value
Subjects included[a]	4	69		7	84		9	75	
Log GMT S0[b]	0.96	1.10	n.s.	1.27	1.07	n.s.	1.25	1.29	n.s.
Response rate (number, %)	3(75%)	46(69%)	n.s.	6(86%)	33(39%)	<0.05	5(56%)	42(56%)	n.s.
Protection rate (number, %)	3(75%)	45(65%)	n.s.	6(86%)	17(20%)	<0.001	3(33%)	20(27%)	n.s.
MFI ± s.d.	1.33 ± 0.54	1.08 ± 0.73	n.s.	1.04 ± 0.63	0.54 ± 0.49	<0.05	0.98 ± 0.79	0.73 ± 0.62	n.s.

[a,b]See *Table 1*. [c]Age cohorts ≤35 and ≥46 years. n.s., not significant

Table 5 Effects of booster vaccination in patients on haemodialysis

Parameter	A-H3N2	A-H1N1	B
Subjects included[a]	71	88	82
Subjects protected after booster/subjects unprotected after first vaccination	7/24(29%)	5/66(8%)**	9/60(15%)
Subjects responding after booster/subjects not responding after first vaccination	10/22(45%)	7/49(14%)**	–/35(0%)***

[a]Number of subjects after exclusion of those with protective prevaccination titres (≥100, influenza A; ≥200, influenza B). In total, 98 HD patients received a booster vaccination. Significant differences between groups: $^{**}p < 0.01$; $^{***}p < 0.001$ versus A-H3N2

other two vaccine components, there were no significant differences between the 36–45 year cohort and the other ages.

Effects of booster vaccination

Additional effects of boosting were estimated in those subjects who, after first vaccination, did not respond or did not achieve protective titre levels. As these subjects were scarce in the control and PD group, because of the high primary response and protection rates (*Table 1*), analysis was done only for HD patients who had received a booster vaccination (*Table 5*). The results were highly dependent on the vaccine components. While the booster effect was poor with regard to A-H3N2, revealing protection in only 29% of previously unprotected subjects and response in only 45% of previously unresponding subjects, it was even significantly less for A-H1N1 (8 and 14%) and B (19 and 0%). Age, sex, prevaccination titres (S0), etiology of renal disease or duration of haemodialysis did not influence the boosting effect.

Discussion

In this study, persons with high prevaccination titres resulting from possible earlier vaccinations or recent natural infections were excluded because of the poor or absent additional increase of titres in this group[21,22]. Moreover, it is of major clinical interest to which extent immunization leads to a protective state in previously unprotected individuals.

Our results, based on a large number of participants, evidently suggest an impaired humoral response to trivalent influenza vaccine in patients on intermittent maintenance haemodialysis which cannot be satisfactorily compensated by a booster injection of the same antigen dosage one month later. This is in accordance with Cappel *et al.*[14], but not with Jordan *et al.*[8], Briggs *et al.*[6], Osanloo *et al.*[5] and Winer *et al.*[7]. However, the number of patients in those studies was small and subjects with high prevaccination titres were included. This might result in underscoring statistically significant differences (type 2 error), as has been demonstrated by Gross *et al.*[23] for studies dealing with a similar issue (influenza vaccination in cancer patients). On the other hand, the comparison with vaccination trials performed in different years should be made cautiously, since the prevalence of prevaccination antibodies against vaccine components, which is a potent factor influencing the seroresponse to vaccination, is annually changing, dependent on the occurrence of natural influenza seasons.

Patients on peritoneal dialysis, even those with short duration of dialysis, showed a significantly better response than patients on haemodialysis. This issue will be discussed in detail elsewhere.

The duration of haemodialysis treatment was not an important factor influencing the seroresponse. Thus, the initially poor humoral response of renal endstage patients when starting haemodialysis, is not worsened or improved by the treatment within the first 8 years. In six of seven HD patients with a treatment longer than 8 years, a significant 'recovery' of seroresponsiveness was established. This issue should be subject to longitudinal studies including more patients.

In HD patients, the three vaccine components showed remarkable differences in evoking seroresponse. The production of anti-A-H3N2-antibodies was least impaired after both first and booster vaccination. The influenza B and, even more pronounced, the influenza A-H1N1 vaccine component caused less and smaller titre rises, as compared to A-H3N2, after first vaccination, and had virtually no effect as a booster. This could be due to low immunogenicity or an insufficient dosage of these two vaccine components or to a laboratory artifact (low avidity). However, the control subjects revealed results for A-H1N1 and B comparable to the A-H3N2 vaccine component. Alternatively, the discrepancy between A-H3N2 and A-H1N1 can be explained by a failure of the primary response in HD patients. Influenza A subtype H3N2 has been circulating since 1968 causing several epidemics with great impact. It is likely that most of the HD patients have been exposed to A-H3N2 strains before starting the dialysis treatment and even before developing severe renal failure impairing the immune functions. Therefore, the vaccination with the recent A/Philippines/2/82(H3N2) virus, except for its new and strain-specific epitopes, is expected to cause an essentially secondary response. Influenza A-H1N1 strains related to the vaccine component A/Chile/1/83(H1N1) circulated in 1947–1957. People born between 1939 and 1949 had most frequently experienced their first influenza A infection by those strains which resulted in a potent immunological memory for that particular subtype ('priming')[24]. It could be shown that this age cohort more frequently produced a typical secondary response to a recent A-H1N1 vaccine than the other ages, even after three decades[25]. Of the HD patients in this study, in particular this age cohort (36–45 years) reacted well against the A-H1N1 vaccine component. In the other age groups, who had not been primed by A-H1N1, the reappearance of this subtype in 1977 did not produce a significant immunological memory because of its small impact in The Netherlands until now. Moreover, most of the HD patients had already been in an altered immunological state as a result of developing uraemia at that time. The data suggest that the A-H1N1 vaccine component served as a primary antigen in HD patients younger than 36 and older than 45 years and did not evoke optimal response. This would be compatible with

findings of Boulton-Jones *et al.*[26] and Byron *et al.*[27,28] suggesting that primary humoral response is profoundly depressed in uraemic patients, while secondary response is less depressed. This conclusion is supported by the results of the booster vaccination: in HD patients who had not responded to the first immunization, a better result was gained against the A-H3N2 component as compared to A-H1N1.

The HD patients of this study are comparable with several other subpopulations within the influenza risk group who do not produce protective levels of anti-HA-antibody. Beside uraemic and renal transplant patients treated with cyclosporin A[15], these include persons of very high age[29,30] and patients with cancer (for review see [23]). The consequences of these findings for vaccine policy in HD patients are unclear. More frequent booster vaccinations or increased dosages are, in theory, useless in a failing primary humoral immune system. We believe that as long as the causes of depressed humoral response in these patient groups cannot be eliminated, the use of amantadine during influenza A epidemics[31] and research on live vaccines[32] and on new generations of vaccine adjuvants[33] should be stimulated.

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