Lack of Effect of a Booster Dose of Influenza Vaccine in Hemodialysis Patients

Elisabetta Tanzi,1* Antonella Amendola,1 Elena Pariani,1 Alessandra Zappa,1 Daniela Colzani,1 Franco Logias,2 Angelo Perego,3 and Alessandro R. Zanetti1

1Department of Public Health-Microbiology-Virology, University of Milan, Via Pascal, Milan, Italy
2Nephrology and Dialysis Service, Sorgono-Isili Hospital, Nuoro, Italy
3Nephrology and Dialysis Service, Nostra Signora di Bonaria Hospital, San Gavino Monreale (Cagliari), Italy

To assess whether the administration of a booster dose of influenza vaccine may enhance immune response in hemodialysis patients, 58 subjects were given two doses of the 2003/2004 season influenza vaccine, 1 month apart. “European Agency for the Evaluation of Medicinal Products” (EMEA) criteria were fully met in terms of percentage of response and of mean-fold increase of hemagglutination inhibiting (HI) antibody titer, but not in terms of seroprotection rates (HI antibody titers ≥1:40). The second vaccine administration did not result in additional increase in seroprotection rate or in geometric mean titers. Protective immune response against the epidemic A/H3N2 Fujian-like strain, antigenically distant from that included in the vaccine (A/Panama/2007/99) was observed in 94.7% of vaccinees protected against the A/H3N2 vaccine strain 1 month after immunization. No adverse reactions were reported during follow-up. The study findings suggest that immune response to influenza vaccination may be suboptimal in hemodialysis patients and that the administration of an additional second dose of vaccine does not improve the humoral response. J. Med. Virol. 79:1176–1179, 2007. © 2007 Wiley-Liss, Inc.

KEY WORDS: influenza; adjuvanted vaccine; hemodialysis patients; hetero-variant

INTRODUCTION

Bacterial and viral infections are a major cause of morbidity and mortality in long-term hemodialysis or peritoneal dialysis patients [Kessler et al., 1993; Khan and Catto, 1993]. Increased susceptibility to these infections is attributed to alterations of the immune system [Descamps-Latscha and Herbelin, 1993; Haag-Weber and Horl, 1993; Girndt et al., 2001].

Since influenza infection can result in severe illness and complications [Rangel et al., 2000], the Advisory Committee on Immunization Practices (ACIP) has stated that chronic dialysis patients may benefit from influenza vaccination and recommends its administration to all chronic renal disease patients aged 6 months or older [Centers for Disease Control and Prevention, 1999]. Several studies reported that in dialysis patients response to influenza vaccination may be suboptimal [Cappel et al., 1983; Vogtlander et al., 2004]. It is yet unclear whether these patients require a higher dosage or increased number of doses to enhance immune response and achieve better protection [Versluis et al., 1987].

In this study, hemodialysis patients were immunized with two doses of a MF59 adjuvanted vaccine licensed for the 2003/2004 influenza season in the northern hemisphere. Concerns were raised regarding the possible reduced protection conferred by this vaccine, as the H3N2 strain (A/Panama/2007/99) used for immunization was antigenically distant from the A/Fujian/411/2002 strain circulating during the following seasonal epidemic [Centers for Disease Control and Prevention, 2004].

The aim of the study was to assess whether the administration of a second dose of influenza vaccine would enhance the immune response in these patients and to determine whether circulating antibodies cross-reacted with the mismatched A/Fujian virus strain.

MATERIALS AND METHODS

Study Population

In November 2003, 58 hemodialysis patients (35 males, 23 females; mean age ± SD: 65.3 ± 13.5 years) attending two Nephrology and Dialysis Units in
Sardinia (Italy) were immunized with two doses of influenza vaccine administered 1 month apart.

Serum samples were collected from each vaccinee prior to (T0), 1 month after the first immunization (T1) and 1 month after the booster injection (T2), and stored at −80 °C until serological analysis.

To allow for evaluation of the vaccine immunogenicity in accordance with the "European Agency for the Evaluation of Medicinal Products" (EMEA) criteria (EMEA, 1997), patients were divided into two age groups: adult vaccinees aged up to 60 years (19 subjects: 11 males, 8 females; mean age ± SD: 49 ± 8.9 years) and elderly vaccinees with age exceeding 60 years (39 subjects: 24 males, 15 females; mean age ± SD: 73.2 ± 6.2 years). Informed consent was obtained from each subject. No participant had contraindications for vaccination as detected by clinical examination or medical history.

Vaccination

Patients enrolled in this study were immunized with a trivalent subunit MF59-adjuvanted influenza vaccine—season 2003/2004—(Fluad®, Chiron Corporation, Emeryville, CA). Injections were administered intra-muscularly in the deltoid region.

The vaccine contained 15 μg hemagglutinin of each of the following viral strains: B/Hong Kong/330/2001 (B), A/New Caledonia/20/99 (A/H1N1), and A/Panama/2007/99 (A/H3N2). Matching between the vaccine strains and the circulating viruses was incomplete, as an A/H3N2 Fujian-like variant (A/Christchurch/28/2003) was predominant during the 2003/2004 influenza season in Italy [Ansaldi et al., 2005].

Humoral Immune Response

Serum samples collected from each vaccinee were assessed for hemagglutination inhibiting (HI) antibodies by standard microtiter assays [Dowdle et al., 1979] for each of the three vaccine influenza strains (A/New Caledonia/20/99, A/Panama/2007/99, and B/Hong Kong/330/2001).

Pre- and post-vaccination sample pairs from each subject were assessed simultaneously. HI antibody titer was expressed as the reciprocal of the highest dilution that inhibited agglutination. Minimum response was defined as seroconversion or as fourfold or greater increase in antibody titer in post-immunization samples. Antibody titers ≥1:40 were considered protective against infection. To calculate the HI geometric mean titer (GMT), a titer of 1:5 was arbitrarily assigned to non-responder vaccinees.

Post-vaccination serum samples from hemodialyzed vaccinees with protective antibody titers (≥1:40) against the homologous A/H3N2 vaccine strain at 1 month from immunization were further tested for the presence of HI antibodies against the epidemic A/H3N2 drifted strain isolated in the 2003/2004 season.

**Statistical Analysis**

Seroconversion rates and proportions of vaccinated individuals with protective HI antibody titers were compared using the Chi square test ($\chi^2$). Student’s $t$-test was used to compare geometric mean HI antibody titers.

**RESULTS**

No adverse reactions were reported following vaccination.

Pre- and post-vaccination serum samples were tested against each of the three vaccine antigens.

One month after the administration of the first vaccine dose (T1), the percentages of response to each antigen were similar ($P > 0.05$) in both the adult and elderly vaccinee groups. These percentages did not differ from the baseline antibody titers measured 1 month after the first injection (T1).

**TABLE I. Percentage of Response (N) to Influenza Vaccination in Hemodialysis Patients**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Adult vaccinees ≤60 years (N = 19)</th>
<th>Elderly vaccinees &gt;60 years (N = 39)</th>
<th>Total vaccinees (N = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
</tr>
<tr>
<td>A/H1N1</td>
<td>57.9 (11)</td>
<td>63.2 (12)</td>
<td>33.3 (13)</td>
</tr>
<tr>
<td>A/H3N2</td>
<td>47.4 (9)</td>
<td>42.1 (8)</td>
<td>35.9 (14)</td>
</tr>
<tr>
<td>B</td>
<td>42.1 (8)</td>
<td>57.9 (11)</td>
<td>46.2 (18)</td>
</tr>
</tbody>
</table>

**TABLE II. Percentage of Vaccinees (N) With Protective HI Antibody Titers (≥1:40) After Influenza Vaccination**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Adult vaccinees ≤60 years (N = 19)</th>
<th>Elderly vaccinees &gt;60 years (N = 39)</th>
<th>Total vaccinees (N = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>A/H1N1</td>
<td>52.6 (10)</td>
<td>94.7 a,b (18)</td>
<td>84.2 (16)</td>
</tr>
<tr>
<td>A/H3N2</td>
<td>42.1 (8)</td>
<td>84.2 a,b (16)</td>
<td>89.5 (17)</td>
</tr>
<tr>
<td>B</td>
<td>5.3 (1)</td>
<td>36.8 a (7)</td>
<td>36.8 (7)</td>
</tr>
</tbody>
</table>

*P < 0.01 and $^b P < 0.05$; T1 versus T0 in both groups of vaccinees.

$^b P < 0.05$; adult vaccinees versus elderly vaccinees at T1.
change significantly ($P > 0.05$) 1 month after the administration of the second dose (T2) (Table I).

Following the first administration of vaccine (T1), a significant increase was observed in the percentage of subjects with protective antibody titers ($\geq 1:40$) for all antigens ($P < 0.01$ for A/H1N1 and A/H3N2 antigens, $P < 0.05$ for B) in both groups of vaccinees. The percentage of vaccinees with protective antibody levels for A/H1N1 and A/H3N2 antigens was higher ($P < 0.05$) in the group of adult compared to elderly subjects. The administration of the booster dose was not followed by a further increase ($P > 0.05$) in seroprotection rate (Table II).

As illustrated in Table III, GMT against each antigen increased significantly ($P < 0.05$) in both adult (mean increase: 2.9–4.6) and elderly vaccinees (mean increase: 2.5–2.8) 1 month after the first immunization without additional significant increase after the second administration.

Finally, 36 out of 38 (94.7%) vaccinees who had protective levels ($\geq 1:40$) of HI antibodies against the homologous A/H3N2 vaccine strain at T1 also showed antibodies protecting against the A/Fujian drifted viral strain. GMT of HI antibody against the homologous vaccine strain was higher than that measured against the heterovariant A/Fujian strain (160.5 vs. 75.7; $P < 0.05$).

### DISCUSSION

In hemodialysis maintenance patients response to influenza vaccination has been shown to be suboptimal [Cappel et al., 1983; Vogtlander et al., 2004], even though several studies reported responses that were almost comparable to healthy controls [Antonen et al., 2000; Brydak et al., 2000]. Moreover, it is yet unclear whether a booster dose can further enhance the level of protection in these patients.

This study evaluated the humoral immune response to two doses of the 2003/2004 season influenza vaccine in 58 hemodialysis patients (19 adults and 39 elderly). Vaccination was safe and no severe adverse reactions were reported among recipients.

The first immunization was followed by a significant increase in the percentage of vaccinees with protective antibody titers ($\geq 1:40$) as well as in GMTs for A/H1N1, A/H3N2, and B antigens in both adult and elderly subjects. As previously shown by Song et al. [2006], no further increases were observed in antibody GMTs or in seroprotection rate after the administration of an additional dose.

A substantial proportion (94.7%) of vaccinees with protective antibody titers against the homologous A/H3N2 vaccine strain at T1 exhibited protective antibodies also against the mismatched A/Fujian/411/2002 strain, considered the major etiological cause of the 2003/2004 season influenza epidemic [Centers for Disease Control and Prevention, 2004]. This finding supports a previously published study which demonstrated that the MF59-adjuvanted vaccine was able to induce cross-reacting antibodies against drifted viral strains, which are not fully matched with those included in the vaccine [Del Giudice et al., 2006]. However, since the A/Fujian drifted strain started to spread in Italy during the spring previous to the study (March 2003) [Ansaldi et al., 2005], it cannot be excluded that the study population might have been partly primed through viral exposure prior to the vaccination.

The clinical acceptability of the vaccine was evaluated using the three immunogenicity endpoints required by the Committee for Proprietary Medicinal Products of EMEA for adult and elderly subjects [EMEA, 1997]. EMEA criteria were fully met in terms of response rate and of mean-fold increase of HI antibody titers, but not in terms of seroprotection rates. Moreover, seroprotection prerequisites were not met even after the administration of an additional dose of vaccine.

Evidence that influenza vaccination is effective in reducing mortality and hospitalization rates among patients on hemodialysis maintenance strongly supports the benefit of vaccination in these patients [Ahmed et al., 1995; Gilbertson et al., 2003]. Therefore, influenza vaccination is widely recommended to hemodialysis patients notwithstanding the suboptimal immune response.

The results of this study indicate that a booster dose of the vaccine does not improve the humoral response to vaccination, but larger studies may be required to clarify this issue.

### REFERENCES


