Immunogenicity of a 2009 Pandemic Influenza Virus A H1N1 Vaccine, Administered Simultaneously With the Seasonal Influenza Vaccine, in Children Receiving Chemotherapy

Gábor Ottóffy, MD,1* Petra Horváth, MD,1 Lajos Muth, MD,1 Alexander Sólyom, MD,1 Miklós Garami, MD, PhD,2 Gábor Kovács, MD, PhD,2 Tibor Nyári, PhD,3 Dénes Molnár, MD, PhD,1 Gábor Pauler, PhD,4 and István Jankovics5

INTRODUCTION

Seasonal influenza viruses and the pandemic influenza virus A (2009 H1N1) have caused significant morbidity and mortality around the world. In most patients, infection results in influenzalike symptoms without complications. In patients with cancer, influenza infection can result in prolongation of chemotherapy, and can cause serious complications, even death [1–7]. A possible option to prevent influenza infection and its complications, is vaccination.

There is conflicting data regarding the effectiveness of seasonal influenza immunization in children during chemotherapy [8–26]. Randomized controlled trials (RCTs), and controlled clinical trials (CCTs), in which the serologic response to influenza vaccination of children with cancer was compared to other control groups, were analyzed in 2009. A more sufficient immune response was generally found in studies in which the majority of children had completed chemotherapy more than 1 month before vaccination [25]. After 2009, few studies investigated the immune response and safety of vaccines for the pandemic influenza virus A (2009 H1N1) in children with malignancy [26–28]. This prospective study evaluates the immune response and safety of concomitant trivalent-inactivated vaccine for seasonal influenza viruses (H1N1A, H3N2A, and B), and monovalent-inactivated vaccine for the 2009 pandemic influenza virus A in children undergoing chemotherapy.

METHODS

Patients

We enrolled 1–18-year-old children receiving chemotherapy for various types of cancer at the Pediatric Oncohematology Units of the University of Pécs and Semmelweis University of Budapest, from November to December, in the years 2009 and 2010. The children enrolled for the study were the consecutive patients treated in the respective centers. We excluded children with a recent history of 2009 pandemic A vaccination, confirmed diagnosis of the pandemic influenza A (2009 H1N1) virus infection prior to the vaccination, and past history of allergy to eggs, as well as those who were receiving other vaccines during the study period.

Study Design

The vaccination schedule was established according to the recommendation of the Infectology Department of the Hungarian National Healthcare Advisory Board, based on data from the Hungarian National Center of Epidemiology. The protocol for this study was approved by the Institutional Review Boards of the University of Pécs and Semmelweis University, Budapest. Sample collections were performed along with the rutin blood sampling involved in cancer treatment. Written consent was obtained from the parents of each child.

© 2014 Wiley Periodicals, Inc.
DOI 10.1002/pbc.24893
Published online 3 January 2014 in Wiley Online Library (wileyonlinelibrary.com).

Background. No examination of simultaneous vaccination against pandemic H1N1 and the seasonal influenza virus strains, in children with cancer receiving chemotherapy, are yet published. We investigated the immunogenicity of a whole-virion, inactivated, adjuvanted pandemic H1N1, and seasonal influenza vaccines administered simultaneously to children with cancer undergoing chemotherapy. Procedure. We prospectively enrolled 27 pediatric patients receiving therapy for various types of cancer. All received influenza vaccination once in a seasonal risk period. We checked hemaglutination-inhibition (HAI) antibody titers in the sera of patients before, and 21–28 days after vaccination. Seroprotective titer was defined as an antibody titer ≥40, and seroresponse as ≥4-fold increase in antibody titers after vaccination. Results. The pre- and post-vaccination seroprotective rates were H1N1: 33–48%, H3N2: 56–77%, B: 0–15% for seasonal influenza, and for pandemic H1N1: 15–37%. The seroresponse rates for seasonal influenza H1N1, H3N2, and B were 22%, 37%, and 22%, respectively, and 30% for the pandemic H1N1 vaccine. Conclusions. Whole-virion, inactivated, adjuvanted vaccine for the pandemic H1N1 Influenza A virus and the seasonal influenza vaccines were found safe and partially immunogenic in children with cancer receiving chemotherapy. The only determinants of responsiveness were lymphocyte count and serum immunoglobulin-G. Only influenza B vaccine elicited significant differences in differences in pre- and post-vaccination seroprotective rates. The response to vaccination for pandemic H1N1 is as effective as other vaccines, however administration of a single vaccine during chemotherapy is more comfortable for pediatric cancer patients. Pediatr Blood Cancer 2014;61:1013–1016. © 2014 Wiley Periodicals, Inc.
Vaccine and Schedule

In 2009, the pandemic vaccine (Fluval P-Omninvest, Pilisborosjenő, Hungary) was a monovalent vaccine (with 6 μg hemagglutinin per 0.5 ml and aluminum phosphate gel adjuvant), the seasonal influenza vaccine (Fluvax AB7-Omninvest) was a trivalent inactivated whole-virion influenza vaccine [29,30]. In 2010, a polyvalent vaccine containing both the seasonal and the pandemic strains, was produced by a method which met the requirements of the European Agency for the Evaluation of Medicinal Products for interpandemic influenza vaccines [30]. The reassortant virus vaccine strain A/California/07/2009 H1N1-like NYMC X-179A was developed from an A/California/7/2009 H1N1 virus [31]. The virus strains of the seasonal vaccine (A/Brisbane/59/2007 H1N1-like; A/Brisbane/10/2007 H3N2-like; and B/Brisbane/60/2008-like strains) were chosen according to the European Union recommendations for the seasonal influenza vaccine composition for the season 2009–2010 [32]. It contained 15 μg hemagglutinin per strain per dose.

In 2009, for children 10 years old and older, 0.5 ml of the pandemic vaccine (monovalent) was injected into the left deltoid muscle, and 0.5 ml of the seasonal vaccine (trivalent) into the right deltoid muscle. In 2010, for children for children 10 years old and older, 0.5 ml of the combined (pandemic and seasonal vaccine—polyvalent) was administered on the left side. In children younger than 10 years old, 0.25 ml was injected.

Vaccination schedule was determined according to the recommendations of the Infectology Department of the Hungarian National Healthcare Advisory Board, based on data from the National Center of Epidemiology, Budapest, Hungary [33]. Vaccination was performed 3–4 weeks after the last chemotherapy administration, and at least 2 days before the next chemotherapy treatment.

Sample Collection and Serologic Analysis

Once informed consent was obtained, a 3-ml blood sample was taken from each participant before vaccination and another 3–4 weeks afterwards, mostly via central venous catheter. The serum was separated by centrifugation, then immediately frozen and stored at −80°C until the laboratory measurements on hemagglutination-inhibition (HAI) antibody titers were performed. Serum antibody titers against the vaccine virus strain were measured by hemagglutination inhibition with chicken red blood cells following standard procedures [34]. All serologic tests were done at a single central laboratory (Department of Virology, National Center of Epidemiology, Budapest, Hungary). All paired sera were tested in duplicate on the same day using identical reagents. Seroprotective titer was defined as having a HAI antibody titer ≥40, and seroresponse was defined as having a fourfold or greater increase in HAI antibody titers after vaccination. Before vaccination, and on day 21–28, blood samples were taken for hemagglutination-titer, immunoglobulin level, white blood cell count, and flow-cytometric analysis.

Adverse Reactions and Medical Conditions

Baseline assessments on day 0 included demographic data, medical history, and physical examination. We checked all patients at least weekly by physical and laboratory (complete blood count) examination during the follow-up period, which lasted until the final blood collection for serologic study. Possible vaccine-related adverse events were monitored. On day 21–28, standard medical history and medications used during the days since the last visit were summarized, and physical examination was done before blood samples were collected. Safety variables were collected at follow-up visits through patient history and physical examination.

Statistical Analysis

Data were analyzed using paired and Student’s t tests, chi-square test (or Fisher exact test) in the univariate analysis. Then the mixed model was applied to investigate the relationship between the outcome of vaccination (seroprotection/seroconversion) and the measured factors (immunoglobulin level, lymphocyte count, age).

For each test, P-value <0.05 was considered significant. All statistical operations were performed using Statistical Package for Social Sciences (SPSS version 20.0).

RESULTS

Demographic Characteristics

Twenty-seven pediatric patients with cancer (15 males and 12 females) completed the study. Their median age was 10.4 years (range 2.83–18.16 years). The underlying diseases were leukemia (10 patients), lymphoma (2 patients), and solid tumor (15 patients). Fourteen patients were younger than 10 years old, and therefore received 0.25 ml of vaccine, while the remaining 13 patients received 0.5 ml of vaccine. All 27 patients received chemotherapy within 1 month before vaccination, and all continued to receive scheduled chemotherapy after vaccination. Five were undergoing maintenance therapy. The remaining 22 were receiving intensive cytostatic treatment. In the latter cases, cytostatic treatment was administered only on the 3rd day after vaccination (Table I).

Seroprotective and Seroresponse Rates

As shown in Table II, pre-vaccination seroprotective rates were significantly higher for seasonal influenza viruses H1N1 and H3N2.

### TABLE I. Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Proportion (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15/27 (55.5)</td>
</tr>
<tr>
<td>Female</td>
<td>12/27 (44.5)</td>
</tr>
<tr>
<td><strong>Age (year)/amount of vaccine</strong></td>
<td></td>
</tr>
<tr>
<td>≤10 year/0.25 ml</td>
<td>13/27 (48.1)</td>
</tr>
<tr>
<td>&gt;10 year/0.5 ml</td>
<td>14/27 (51.9)</td>
</tr>
<tr>
<td><strong>Cancer type</strong></td>
<td></td>
</tr>
<tr>
<td>Hematological malignancies</td>
<td>12/27 (44.5)</td>
</tr>
<tr>
<td>Solid tumors</td>
<td>15/27 (55.5)</td>
</tr>
<tr>
<td><strong>Treatment type at first vaccination</strong></td>
<td></td>
</tr>
<tr>
<td>Intensive</td>
<td>22/27 (18.5)</td>
</tr>
<tr>
<td>Maintenance</td>
<td>5/27 (18.5)</td>
</tr>
<tr>
<td><strong>Lymphocyte count</strong></td>
<td></td>
</tr>
<tr>
<td>Lymphopenic (≤1.0 G/L)</td>
<td>6/26 (23.0)</td>
</tr>
<tr>
<td>Not lymphopenic (&gt;1.0 G/L)</td>
<td>20/26 (77.0)</td>
</tr>
<tr>
<td><strong>IgG level</strong></td>
<td></td>
</tr>
<tr>
<td>≤Age related normal</td>
<td>5/19 (26.3)</td>
</tr>
<tr>
<td>Age related normal</td>
<td>14/19 (73.7)</td>
</tr>
</tbody>
</table>

*Lymphocyte count on day 0 failed in one case; †IgG level on day 0 unavailable in six cases.*

Pediatr Blood Cancer DOI 10.1002/pbc
H1N1, H3N2, B are seasonal influenza virus strains, and H1N1Swl is the pandemic influenza virus.

Table II. The Pre- and Post-Vaccination Seroprotective Rates, and Seroresponse Rates for Seasonal Influenza Virus Strains and for the Pandemic H1N1Swl

<table>
<thead>
<tr>
<th></th>
<th>H1N1</th>
<th>H3N2</th>
<th>B</th>
<th>H1N1Swl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-vaccination seroprotective rates</td>
<td>9 (33.33%)</td>
<td>15 (55.55%)</td>
<td>0 (0%)</td>
<td>4 (14.8%)</td>
</tr>
<tr>
<td>Post-vaccination seroprotective rates</td>
<td>13 (48%)</td>
<td>21 (77.78%)</td>
<td>4 (14.8%)</td>
<td>10 (37%)</td>
</tr>
<tr>
<td>Post-vaccination seroresponse rates</td>
<td>6 (22.22%)</td>
<td>10 (37%)</td>
<td>6 (22.22%)</td>
<td>8 (29.62%)</td>
</tr>
</tbody>
</table>

H1N1, H3N2, B are seasonal influenza virus strains, and H1N1Swl is the pandemic influenza virus.

than for seasonal B and pandemic H1N1Swl (Fisher exact \( P < 0.001 \)). The differences between pre- and post-vaccination seroprotective rates in the case of Influenza B were significant \( (P = 0.038) \). No significant differences were found between pre- and post-vaccination seroprotective rate changes with other viruses (H1N1Swl \( P = 0.064 \), H1N1 \( P = 0.277 \), H3N2 \( P = 0.160 \)). We compared the pre- and post-vaccination seroprotective rate changes and seroresponse rate of each seasonal Influenza virus with the pandemic H1N1Swl virus by a paired \( t \)-test. Neither HAG seroprotective rate changes, nor seroconversion for seasonal H1N1–H1N1Swl, H3N2–H1N1Swl, B–H1N1Swl were significant using this method.

Comparing the effect on seroprotection change and seroconversion of each virus type combined with age category (over and below 10 years) using binary logistic regression, there was no significant difference either in seroprotection rate changes or in seroconversion, between the different influenza virus strains. Lymphocyte counts at the time of vaccination were between 0.44 and 7.77 G/L (median: 1.34 G/L). Lymphocyte count above 1.0 G/L was significantly correlated with immune response after influenza vaccination \( (P = 0.018) \). On the other hand, lymphocyte count above 1.0 G/L did not have a significant influence on seroconversion change. These results were independent of virus strain. The immunoglobulin G (IgG) levels at the time of vaccination were between 4.27 and 12.8 g/L (median: 7.32 g/L). Most (14/19) of the patients, had age related normal level of IgG at the time of vaccination. This was significantly correlated with immune response after influenza vaccination, independently of virus strain \( (P = 0.01) \). Age related normal IgG level did not have a significant influence on seroprotection change \( (P = 0.063) \). Type of malignancy and status of cancer therapy (maintenance or intensive treatment) did not significantly influence seroprotection change or seroconversion. There were no breakthrough influenza infections, and neither of the nonvaccinated patients suffered from influenza during this period.

Adverse Reactions

We recorded no local adverse reactions (e.g., injection site induration, erythema, swelling, or warmth). One case of malaise (3.7%) was detected on the day after vaccination. No medical intervention was necessary. No vaccine related serious adverse events were observed.

DISCUSSION

The purpose of this study was to investigate the efficacy and safety of concomitant trivalent-inactivated vaccines for seasonal influenza viruses (H1N1A, H3N2A, and B), monovalent-inactivated vaccine for the 2009 pandemic influenza virus A, and a combined (pandemic and seasonal vaccine—polyspecific) vaccine in 2010, in children with cancer undergoing chemotherapy. Vaccinations (H1N1A, H3N2A, B, and pandemic H1N1Swl) produced no significant adverse events, but resulted in limited seroresponse rates (22%, 37%, 22%, and 30%, respectively). These results were similar to response rates reported earlier for influenza viruses [8–26], and recently for pandemic influenza virus A [26–28].

Pre-vaccination seroprotective rates were significantly higher for seasonal influenza viruses H1N1A and H3N2A, than for seasonal B and pandemic H1N1Swl viruses (Table II). A possible explanation is that a significant time elapsed since the last Influenza B epidemic (2004–2005) affecting our region, while the H1N1Swl pandemic started at the time of vaccination in our study. The four detected seroprotective titers for pandemic H1N1Swl virus were all from samples obtained in 2010, and all patients had received a vaccination one year earlier. On the other hand, Influenza virus H3N2A is highly immunogenic, and seasonal Influenza virus H1N1A has a high circulate tendency.

Our patients were receiving chemotherapy at the time of vaccination, thus limited seroprotection changes and seroresponse rates were expected. The proportion of patients achieving protective antibody levels to individual viral strains following vaccination has been reported to be 29–75% in children on chemotherapy [8,12,23]. A metaanalysis (Cochrane Database Syst Rev. 2013) has reported on the efficacy of influenza vaccination in stimulating an immunological response in children with cancer during chemotherapy, compared with control groups. Immune responses in children receiving chemotherapy were consistently weaker (fourfold rise of 38–65%) than those in children who had completed chemotherapy (50–86%) and in healthy children (53–89%) [35–38]. A pre-pandemic influenza A (H5N1) vaccine, prepared with same method (Fluval-Omninvent) as the vaccine used in this trial, was assessed in healthy children, where the rate of seroconversion was 75% and the rate of seroprotection was also 75%, 21 days after vaccination [39]. In this study, 15–78% of children achieved protective antibody levels to individual viral strains following vaccination. However, post-vaccination seroprotection rates were significantly, or nearly significantly higher than pre-vaccination rates in the case of Influenza virus B (pre-: 0%; post-vaccination: 15%, \( P = 0.038 \)), and pandemic H1N1Swl (pre-: 14.8%; post-vaccination: 37%, \( P = 0.064 \)). The significant difference in seroprotection changes may due not to the type of vaccine, but to the low pre-vaccination titers, specific to the two viruses mentioned above. In the present study, all children were receiving chemotherapy, and an immune response was observed in 22–37% of children. There was no significant difference in either post-vaccination seroprotection change or seroconversion of the different seasonal influenza viruses when compared with the pandemic H1N1Swl virus, results similar to those obtained in healthy people [29].
According to the vaccination recommendations for children below the age of 10 years, half the adult dose was used. Neither the type of vaccine (a trivalent seasonal vaccine and a monovalent pandemic H1N1Sw1 vaccine in 2009, and a polyclonal seasonal and pandemic vaccine in 2010), nor the age of patients, influenced the immune response and immunologic protection in our study. There is controversial data regarding lymphocyte count, and serum IgG level [8,9,12,13,14,15,26], as factors responsible for immune response after administration of influenza vaccine in children with cancer undergoing chemotherapy. The present study demonstrates that, for both seasonal viruses and the pandemic H1N1 virus, lymphocyte count above 1.0 G/L, and normal IgG level has significant influence on the immune response after vaccination. The immunoglobulin G (IgG) levels at the time of vaccination were in the normal range in most of our patients. One of the possible explanations of the normal values among patients treated with chemotherapy is, that half of the patients with the leukemia were already on maintenance therapy. On the other hand most of our patients had solid tumors. These underlying diseases in general do not affect the immunsystem and their treatment is often less myelosuppressive.

Several reports suggest that a two-dose influenza vaccination series produces a better immune response than a one-dose vaccine for oncology patients receiving chemotherapy [8,11,12,13,15,23]. However, a recent comparison of the rates of seroconversion and seroprotection between patients who received two doses of H1N1 vaccine, and those who received one dose, did not show any significant difference [26]. In our study, we could only compare our results to those of other similar studies, being that we used a one-dose vaccine.

This study has several limitations. The patient sample was quite heterogeneous regarding the specific underlying diseases and type of cytostatic treatment. Statistically the study sample size was small and no control group was established. Therefore our findings should be treated with caution. On the other hand, we tried to counterbalance it with selecting patients into sample mostly received parenteral cytostatic treatment. Moreover, we published only results with p value under 1% and omitted nearly all plausible significant provable findings (1% < P < 5%). Finally, safety measure we compared our findings with literature, whether they confirm mainstream theories.

**CONCLUSION**

This report investigated on vaccination against pandemic H1N1 and the seasonal influenza viruses simultaneously, in children with cancer receiving chemotherapy. We confirmed that present pandemic vaccine can be safely co-administered with the 2009–2010 seasonal influenza vaccine even in this population. There was no significant difference in either post-vaccination seroprotection change or seroconversion of the different seasonal influenza viruses when compared with the pandemic H1N1Sw1 virus. This strategy may enhance seroresponse to influenza vaccination if the absolute lymphocyte count is above 1.0 G/L, and IgG levels are in the age related normal reference range. These results were achieved with single-dose vaccines, which were well tolerated. Randomized control trials with larger sample sizes are needed to determine the optimal influenza vaccination regimen, with regard to timing, dosage, number of doses, and adjuvantation of the vaccine.

**REFERENCES**