Response to influenza virus vaccination during chemotherapy in patients with breast cancer


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Background: Patients receiving chemotherapy are at increased risk for influenza virus infection. Little is known about the preferred moment of vaccination during chemotherapy.

Patients and methods: Breast cancer patients received influenza vaccination during FEC (5-fluorouracil, epirubicin and cyclophosphamide)-containing chemotherapy regimens. Patients were randomised for early (day 4) or late (day 16) vaccination during the chemotherapy cycle. Influenza virus-specific antibody titres were determined before and 3 weeks after vaccination by haemagglutination inhibition.

Results: We included 38 breast cancer patients (20 in the early and 18 in the late group) and 21 healthy controls. The overall patient group had significant lower responses to the vaccine compared with healthy controls. Patients vaccinated at day 4 tended to have higher antibody titres as compared with patients vaccinated at day 16, although the difference in post-vaccination titres is not statistically significant. Geometric mean titres post-vaccination for day 4 versus day 16 were 63.7 versus 29.5 (H3N2), 28.2 versus 19.6 (H1N1) and 29.8 versus 16.0 (B/Brisbane), respectively.

Conclusions: Patients on chemotherapy have significantly lower responses to influenza virus vaccination compared with healthy controls. Vaccination early during the chemotherapy cycle induces better responses than does vaccination at day 16 of the cycle. Follow-up studies are needed to confirm this effect.

Key words: chemotherapy, haemagglutination inhibition, influenza virus vaccination

Introduction

Seasonal influenza virus infections are an important cause of acute respiratory disease [1]. Patients with cancer are at risk for serious post-influenza complications because immunosuppressive factors influence the response to viral infection. The disease state itself is immunosuppressive and the immune response to natural viral infections is impaired by the chemotherapy [2]. Cancer patients are eligible for influenza vaccination, although their response, for reasons outlined above, may be suboptimal [3].

Yearly, 441 per 100 000 oncology patients are hospitalised in the United States because of influenza virus infections, which is 3–5 times higher than in the general population. Moreover, the mortality rate is 9% in oncology patients (relative risk of 4 compared with the general population) [4]. 
effect of the timing of influenza vaccination during chemotherapy in patients with breast cancer.

patients and methods

study design
In the general population, the serological response to the influenza virus vaccination is found to be ~80%. For breast cancer patients, the immunosuppressive effect of the chemotherapy is assumed to be larger early after the chemotherapy than later in the cycle. However, data about the exact percentage of patients with a seroconversion after chemotherapy are scarce. The design of the study is the comparison of the response to influenza vaccination in terms of rise in antibody titre between patients receiving chemotherapy for breast cancer versus healthy individuals. In breast cancer patients on chemotherapy, the response to vaccination is determined in patients vaccinated early (day 4) versus late (day 16) during a chemotherapy cycle.

patients
We included patients who received adjuvant chemotherapy because of breast cancer. All patients were treated with FEC (5-fluorouracil 500 mg/m², epirubicin 100 mg/m² and cyclophosphamide 500 mg/m²) six cycles or FEC three cycles followed by docetaxel (100 mg/m²) three cycles.

Exclusion criteria were fever at time of vaccination (temperature of ≥38.5°C), known allergic reaction to any of the components of the vaccine (e.g. hypersensitivity to egg protein), platelet count <50×10⁹/l at the moment of vaccination or treatment with prednisone at the moment of vaccination.

This study was approved by the central committee on research involving human subjects of the Netherlands (CCMO). All patients signed informed consent. The study was registered at ClinicalTrials.gov with ID number NCT01000246.

This multicenter randomised trial was executed in seven hospitals in the Netherlands. Eligible patients were randomised for early (day 4) or late (day 16) vaccination during a chemotherapy cycle. A randomisation schedule was used in which patients were randomised 1 : 1 with randomisation in blocks of 10 patients. Patients were allocated to either group in order of notification of the principal investigator. The results of vaccination in the patient group were compared with those of vaccinated female employees of one of the participating hospitals. These employees participated voluntarily in the yearly influenza vaccination campaign for health care personnel.

vaccination
In October/November 2009, patients received one of the two available seasonal influenza virus vaccines (one dose of 0.5 ml), dependent on which vaccine was available in the participating hospitals. These two vaccines (Influvac® 2009/2010, Solvay Biologicals B.V, Olst, the Netherlands and Vaxigrip® 2009/2010, Sanofi Pasteur MSD, Brussel, Belgium) each containing 15 μg haemagglutinin of the following influenza strains: A/Brisbane/10/2007 (H3N2)-like strain, A/Brisbane/59/2007 (H1N1)-like strain, B/Brisbane/60/2008-like strain.

laboratory investigations
Serum samples were collected before and 3 weeks after vaccination and stored at −70°C until use. Before vaccination, blood cell counts including leucocyte differentiation were carried out.

Antibodies to the haemagglutinin of all three vaccine strains were measured by the haemagglutination inhibition (HI) test, according to standard procedures using four haemagglutinating units of virus and turkey erythrocytes [9, 10]. Twofold serial dilutions of patients sera were tested with a starting dilution of 1 : 20. Sera were pretreated with receptor-destroying enzyme (cholera filtrate) to remove non-specific inhibitors and then tested for HI antibodies specific for the viruses A/Brisbane/10/2007 (H3N2), A/Brisbane/59/2007 (H1N1) and B/Brisbane/60/2008. The antibody titre is expressed as the reciprocal value of the highest dilution that still inhibits agglutination.

Data are expressed as geometric mean HI titre (HI GMT), seroprotection rate (SPR; i.e. the percentage of vaccine recipients with a serum HI titre ≥40 after vaccination) and seroconversion rate (SCR; i.e. the percentage of vaccine recipients with a fourfold increase or more in post-vaccination titre) [11, 12]. According to the criteria of the EMEA (European Agency for the Evaluation of Medicinal Products) in healthy adults ≥60 years of age, an adequate response to vaccination includes one of the following three requirements of the serological assessments: SPR ≥70%, SCR ≥40% and mean increase in GMT ≥2.5 [13]. In persons older than 60 years, the criteria for an adequate response are SPR ≥60%, SCR ≥30% and GMT ≥2.0, respectively.

statistical analysis
For statistical purposes, HI titres <10 were assigned an arbitrary value of 5. Comparisons of GMT before and after vaccination (paired samples) were carried out using the Wilcoxon signed rank test. For independent samples, the Mann–Whitney U test was used. Pearson’s χ² or Fisher’s exact test were used to compare groups. A P value of <0.05 was considered statistically significant.

results
Forty-four patients with breast cancer fulfilled the inclusion criteria. Five of these patients were excluded at the time of vaccination due to intercurrent infectious diseases (n = 4) or preference for the H1N1 vaccine (n = 1). One patient received the H1N1 vaccination, just before the second serum sample was taken and was excluded from our analysis due to potential interference (Figure 1).

Of the resulting 38 breast cancer patients, 20 patients had been randomised for vaccination on day 4 of the chemotherapy cycle and 18 for vaccination on day 16.

The serological response to vaccination was compared with the response of 24 healthy female employees of one of the participating hospitals. In three persons non-specific erythrocyte agglutination was found, preventing HI activity measurement. Therefore, these patients were excluded. The mean age of the 21 controls used in this study was 35.6 years, range: 24–62 years. Two healthy controls (10%) were >60 years. Nine (43%) controls had been vaccinated against influenza previously (mean of two previous vaccinations).

In Table 1, baseline characteristics of the breast cancer patients are given. Three patients had multiple comorbidities. The two groups did not differ in age, type of chemotherapy (i.e. FEC or FEC-docetaxel), previous influenza vaccination or comorbidity. Haemoglobin, leukocytes and neutrophils were significantly lower in the late vaccination group.

Haemagglutination inhibition titres were measured pre- and post-vaccination. For each influenza strain in the vaccine, antibody titres were determined separately (Figure 2).

The antibody response to influenza vaccination in the patient group as a whole was significantly lower than in the control group. In the early patient group and control group, GMT post-vaccination increased significantly for all three virus strains. In the late patient group, a statistically significant
increase in GMT post-vaccination was only found for the H1N1 virus strain.

No statistically significant differences were found between the pre-vaccination titres in the early and late patient group versus the control group. Numbers of patients with HI titres ≥40 pre-vaccination are given in Table 2. No differences in GMT’s post-vaccination were seen between the two different vaccines that were used (data not shown).

Previous influenza vaccination had a statistically significant influence on the pre-vaccination HI titre of H1N1 in the early patient group (GMT 14.4 in the previous vaccinated group versus 5.4 in the vaccine-naive group, \( P = 0.01 \)). In the control group, previous vaccination had a statistically significant influence on the antibody levels, pre-vaccination to both H3N2 and H1N1 strains (GMT 65.5 versus 13.6, \( P = 0.03 \) and GMT 20.3 versus 5.9, \( P = 0.05 \), respectively).

As can be seen in Table 2, for all three virus strains the control group meets all three criteria of the EMEA for defining an adequate response. In the early patient group, SPR criteria are not met, and SCR >40% is only met for H3N2. In the late patient group, SPR and SCR criteria are not met for any virus strain. A GMT increase >2.5 is found for all three virus strains in the early patient group but not in the late patient group. Although not significant, the post-vaccination titres in the early patient group were overall higher for each virus strain compared with the late patient group.

In our total patient group (\( n = 38 \)), patients older than 60 years had higher pre-vaccination HI titres for the H3N2 virus strain than patients aged 60 years and younger (GMT 43.6 versus 13.3, \( P = 0.04 \)). The HI titre for the B/Brisbane strain, however, was lower in patients older than 60 years (GMT 5.6 versus 10.5, \( P = 0.02 \)). In the overall patient group, all patients older than 60 years had received prior vaccination compared with 17% of the patients aged 60 years and younger (\( P < 0.01 \)).

**Discussion**

We studied the response to the seasonal influenza virus vaccination in patients receiving FEC-containing chemotherapy for breast cancer. Compared with healthy controls, the response was significantly lower in the cancer patients. When comparing both patient groups (early versus late), we observed a trend towards a relatively higher response in the early vaccination group.
Thrombocytes (range), significant; COPD, chronic obstructive pulmonary disease. FEC, 5-fluorouracil, epirubicin and cyclophosphamide; n.s., non-significant.

Comorbidity, n

Previous vaccination, n

Chemotherapy, n

Haemoglobin (range), mmol/l 7.6 (6.4–8.9) 7.2 (6.2–8.1) 0.04

Leucocytes (range), x10^9/l 6.2 (2.4–9.7) 3.4 (1.7–9.4) <0.01

Neutrophils 4.9 (0.7–8.4) 1.7 (0.2–7.3) <0.01

Lymphocytes 1.3 (0.2–4.4) 1.0 (0.4–1.52) n.s.

Thrombocytes (range), x10^9/l 309 (167–532) 272 (127–419) n.s.

Figure 2. Haemagglutination inhibition (HI) antibody titres in patients and controls. Open bars show pre-vaccination GMT, closed bars show post-vaccination GMT. GMT, geometric mean titre. In all cases, vaccination induced a significant increase in GMT, except for the late patients group for vaccination GMT. GMT, geometric mean titre. In all cases, vaccination and controls. Open bars show pre-vaccination GMT, closed bars show post-vaccination GMT.

In healthy adults, inactivated influenza vaccine has a 70%–90% efficacy in preventing influenza in case of a sufficient antigenic match between the vaccine and the epidemic virus [1, 14]. Serum HI antibody titres of ≥40 (SPR) are associated with at least a 50% reduction in risk for influenza infection or disease in populations [15–17].

Patients vaccinated early in the chemotherapy cycle (day 4) showed a trend for higher GMT’s and higher percentages of SPR and SCR than the late patient group. Breast cancer patients had significantly lower responses in SPR, SCR and GMT’s compared with our healthy control group. This is consistent with other studies on the effect of chemotherapy in cancer patients on the response to influenza vaccines [18, 19].

It was previously shown that during chemotherapy, patients with breast cancer indeed had a lower response to vaccination (given 24–48 h before cytotoxic treatment) than patients who did not receive chemotherapy [20]. On the other hand, it has also been reported that the immune response to influenza vaccination in breast cancer patients with or without chemotherapy was as effective as that of healthy adults [2]. The patient group, however, was small (n = 9) and heterogeneous.

Guidelines on the timing of vaccination of oncology patients (both haematological and solid tumours) on chemotherapy are mostly based on a single study in which the outcome of vaccination at the day of chemotherapy or at the time of the white blood cell count nadir were compared [8]. Only 50% of the patients vaccinated at the day of the chemotherapy achieved seroconversion, whereas 93% of the patients vaccinated in their nadir showed seroconversion. It was concluded that vaccines should not be given at the day of chemotherapy. This study was conducted in 1977 and since that time, considerable changes have been made in vaccine formulations and chemotherapy regimens, giving a possible explanation for the difference in response compared with our patient group.

In the current study, a trend was seen towards better responses to vaccination in patients vaccinated early (day 4) compared with vaccination in the last week of the chemotherapy cycle (day 16). This trend may be explained...
by the effect of chemotherapy on the different phases of a humoral response on vaccination.

Although the leucocyte count at baseline was significantly lower in the late vaccination group, absolute lymphocyte numbers did not differ significantly between day 4 and day 16. However, chemotherapy will likely influence the proliferative phase of lymphocytes, a crucial phase of the immune response. In the early patient group, 17 days pass before the next dose of chemotherapy is given, enabling a substantial proliferative phase of the humoral response to take place. In the late group, only 5 days pass before lymphoproliferation is abrogated by the chemotherapy. This scenario would not hold true for patients in the final cycle of chemotherapy. Indeed, in the patients vaccinated at day 16 of the last (sixth) cycle (n = 4), antibody responses were higher compared with patients vaccinated at day 16 during any of the other cycles (n = 14). For H1N1, this difference was statistically significant, with a GMT HI post-vaccination in cycle 6 of 101.9 versus 12.3 in other cycles (P = 0.035). This confirms that the subsequent chemotherapy dose would be responsible for suppression of the antibody response in patients vaccinated at day 16 during chemotherapy.

It should be noted that the lower total leucocyte counts in the late vaccination group may also have contributed to the observed differences in antibody response.

During the design of our study, a pandemic rise in infections with the so-called Mexican flu virus (H1N1) appeared and a national vaccination campaign against this infection was launched. Priority was given to H1N1 vaccination and we therefore had to prematurely stop the inclusion of patients and only 38 patients could be included in this study. Despite the relatively small number of included patients, a clear trend towards better responses in patients vaccinated early in the chemotherapy cycle is seen.

Recently, seasonal influenza virus vaccines with adjuvants became available. The use of an adjuvant enhances immune stimulation, with higher GMT’s post-vaccination, maintaining a trend towards better responses in patients vaccinated early in the chemotherapy cycle.

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**disclosure**

The authors declare no conflict of interest.

**references**


**acknowledgements**

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