Intradermal influenza vaccination in immunocompromised patients is immunogenic and feasible


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ABSTRACT

Background. Many strategies, including intradermal vaccination, have been tested to augment antibody responses upon vaccination. This strategy has not been evaluated in different groups of immunocompromised patients. We conducted a prospective, randomized study to compare the humoral response upon standard intramuscular influenza vaccination with the response upon reduced-dose intradermal vaccination in patients treated with anti-tumor necrosis factor (TNF)-alpha, human immunodeficiency virus (HIV)-infected patients, hematologic stem cell transplantation (HSCT) patients, and healthy controls.

Methods. In total 156 immunocompromised patients and 41 healthy controls were randomized to receive either 0.5 mL of the 2005/2006 trivalent influenza vaccine intramuscular or 0.1 mL intradermal. Humoral responses, determined by hemagglutination inhibition assay, were measured before and 28 days post vaccination. Geometric mean titers (GMTs) and protection rates (PRs) are reported as primary outcomes, adverse events as a secondary outcome.

Results. Reduced-dose intradermal vaccination leads to similar GMTs and PRs, within all tested groups, compared to the standard intramuscular vaccination. Healthy controls yielded significantly better GMTs and PRs than immunocompromised patients. Local skin reactions after intradermal vaccination occurred less frequent and were milder in immunocompromised patients than in healthy subjects and were predictive for a positive vaccination outcome for individual subjects.

Conclusions. Intradermal influenza vaccination is a feasible alternative for standard intramuscular vaccination in several groups of immunocompromised patients, including those treated with anti-TNF, HIV-infected patients and HSCT patients. The occurrence of a local skin reaction after intradermal vaccination is predictive of a response to at least one of the vaccine antigens.
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1. **BACKGROUND**

   Annual influenza vaccination is recommended for immunocompromised patients. [1-4]

   Besides causing a greater risk of complications from influenza infection, an immunodeficiency compromises the response to (T-cell-dependent) vaccine antigens. [5-7] Many strategies have been explored in immunocompromised patients in order to optimize vaccination outcomes, including increased dosage, multiple dose vaccination, the use of vaccine adjuvant, immunostimulant patches and more efficient routes of vaccine delivery. [8-12]

   In persons with impaired immunity intradermal vaccination is of particular interest because of anticipated immunologic advantages. The dermis harbours a network of antigen presenting cells, constituting up to two percent of all dermal cells, which forms an optimal environment to deliver a vaccine. [13-15] Since there is a dose-response relation for antigen quantity and antibody response, studies in immunocompromised patients have mostly concentrated on higher vaccine doses administered intramuscularly. [6, 16] The favourable immunologic properties of the dermis allow for smaller quantities of vaccine to be used. Healthy subjects respond to reduced-dose intradermal influenza vaccination generally just as well as to standard intramuscular vaccination, especially in populations already primed with the antigen. [17-26] Intradermal influenza vaccination received most attention in times of vaccine shortage caused by pandemics (e.g. the 1957 influenza A/H2N2 pandemic) or manufacturing problems as occurred in 1973 and 2004. [27, 28] These studies concentrated on the responses of healthy subjects. Only little information is available on intradermal influenza vaccination in immunocompromised patients. [29]

   Besides a potential immunologic benefit, there is an obvious economic advantage in saving up to 80% of the vaccine required, allowing for full vaccination coverage even in times of vaccine shortages. [30-31] There are however also some inherent practical disadvantages to intradermal vaccination, especially relevant in mass vaccination campaigns such as the annual influenza vaccination campaigns or the response to an eventual pandemic influenza outbreak. The vaccination technique itself is more difficult and time consuming than intramuscular vaccination. Furthermore, local side effects in healthy subjects, are more severe and frequent upon intradermal vaccination when compared to intramuscular vaccination, a factor known to negatively impact vaccine uptake. [32-33] These factors and the small number of data on intradermal vaccination in subjects with an indication for annual influenza vaccination have likely contributed to the decision from the Center of Disease Control and Prevention (CDC) to discourage intradermal influenza vaccination in its 2007 guidelines, especially for persons older than sixty years. [1]

   Before intradermal influenza vaccination can be implemented as a routine procedure, the efficacy and safety should be established in patient groups with an indication for annual influenza vaccination. We conducted a prospective, randomized study to test the
hypothesis whether reduced-dose intradermal influenza vaccination in immunocompro-
mised patients, in particular those treated with anti-tumor necrosis factor (TNF) alpha,
persons infected with the humane immunodeficiency virus (HIV) and patients who have
undergone hematological stem cell transplantation (HSCT), is safe and whether it leads
to an equal quantitative serologic response as compared to the response upon standard
intramuscular vaccination.

METHODS

Trial design and subjects

A group of healthy controls (HC group) and three groups of immunocompromised pa-
tients were included in this trial. Rheumatologic patients with or without other immuno-
suppressives at the Leiden University Medical Center (LUMC), Leiden or the Sint Maartenskliniek, Nijmegen, The Netherlands (anti-TNF group); HIV infected patients in care at the LUMC (HIV group) and HSCT patients treated at the LUMC (HSCT group) were asked to participate.

Importantly, this study was designed to mirror clinical practice, with few limitations
for inclusion. Exclusion criteria included only known allergy to influenza vaccine, severe chicken egg allergy, age below 18 years, the use of oral anticoagulation therapy, a throm-
bocyte count less than 25 x 10⁹ per liter and a skin type not suitable for intradermal vaccine delivery (such as severe corticosteroid-induced skin atrophy). The four study groups were randomized to receive either standard intramuscular vaccination or reduced dose intradermal vaccination. Permuted-block randomization was performed with the use of sealed envelopes containing the balanced vaccination codes for ten subjects. All subjects were asked to register adverse reactions in a study log. The study protocol was approved by the appropriate institutional ethics committees (LUMC, local CME number P05.115; Sint Maartenskliniek local CME number RR-14-GRIEP; ISRCTN15762138) and conducted in accordance with the Declaration of Helsinki. All participants provided a written informed consent.

Vaccine and vaccination

All study subjects were vaccinated in the fall and winter of 2005 with a commercially avail-
able trivalent subunit influenza vaccine (Influvac™ 2005/2006, Solvay Pharmaceuticals B.V., Weesp, The Netherlands) at day 0. The vaccine contained 15 μg of hemagglutinin of each of the following strains: A/California/7/04 (H3N2) like strain (A/New York/55/2004 NYMC X-157 reassortant) further referred to as A/H3N2; A/New Caledonia/20/99 (H1N1) like strain (A/New Caledonia/20/99 IVR-116 reassortant) further referred to as A/H1N1, and B/Shanghai/361/02 like strain (B/Jiangsu/10/03) further referred to as
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1. influenza B. The A/H3N2 was the only new strain in this vaccine: the A/H1N1 vaccine strain remained unchanged since the season of 2000/2001; the influenza B strain was identical to the strain used in 2004/2005.

4. Vaccines were stored at 6°C and administered at room temperature. Intramuscular vaccinations (0.5 mL) were given according to the package insert by injection in the left deltoid muscle. On a daily base vaccine for the intradermal administration was transferred from the pre-filled vaccination syringe into sterile vials and 0.1 mL of vaccine fluid, containing 3 μg of hemagglutinin of the three strains, was then delivered intradermal in the palmar side of the forearm according to the CDC guidelines using an intradermal injection syringe (BD Micro-Fine 0.5 mL U-100 insulin syringe) and needle (29G), also used for intradermal tuberculin injections. [34] All vaccines were administered by the investigators who were trained in both vaccination techniques. Directly after vaccination wheals were measured to confirm adequate intradermal vaccine delivery.

15. Antibody assays and statistics

16. Serum samples were collected at day 0 (before vaccination) and day 28 and were stored at –80°C until use. The hemagglutination inhibition (HI) test was performed in duplicate according to standard methods with turkey erythrocytes and four hemagglutinating units of virus to measure antibodies against each of the three vaccine strains as described before. [5] Ferret sera raised against the test antigens were used as positive controls. All sera of each individual study subject were tested simultaneously. For statistical analysis a titer of 5 was arbitrarily assigned to sera with a titer <10. Titers were transformed to a logarithmic scale and geometric means were used for further calculations. Geometric mean titers are the strongest markers of the immunological capability of a group to respond to an antigen and were therefore preferred over the response rate (fourfold titer increase), or other outcome measurements that depend heavily on prevaccination titers. Protection rates (PR) were defined as the percentage of patients with a HI titer ≥ 40, which is considered to be a clinically relevant titer, known to be associated with protection against severe influenza in healthy controls, after vaccination. [5, 35] The results were analyzed by one-way ANOVA for geometric mean titers (GMTs), two-sided χ²-test for PR.

31. Multivariate analysis of postvaccination GMTs, using a logistic regression model including the variables age, sex, previous vaccination, prevaccination titer and vaccination route was performed on the four different study groups. Group specific variables were added to the model were applicable (DAS28, type of anti-TNF, use of methotrexate in the anti-TNF group; a CD4-count of less than 200 cells per mm³, CD4 nadir, the use of HAART in the HIV group; type of transplantation and being within the first year post transplantation in the HSCT group).

38. A p-value <0.05 was considered to indicate a statistical significant difference between groups. Calculations were performed using SPSS for Windows, version 14.0.
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RESULTS

Baseline characteristics

Baseline characteristics of the 197 participants who were randomized are summarized in Table 1. The 41 healthy controls included many healthy partners of patients and hospital staff. The hospital endorses an active policy of influenza vaccination, explaining the relatively high percentage that received a prior influenza vaccination. Fifty patients treated with anti-TNF (forty RA patients, six with spondyloarthritis, three with psoriatic arthritis and one with juvenile chronic arthritis) were well matched with regard to gender, age, percentage of patients with RA, disease activity score (DAS28), type of anti-TNF used (two-third were treated with etanercept, one sixth each with infliximab and adalimumab) and other immunosuppressives (methotrexate was used by 28% of the patients and prednisone by 12%). The percentage of study subjects in the anti-TNF group vaccinated in previous influenza seasons was almost twice as high in the intradermal group as compared to the intramuscular group (p=0.08). A DAS28 of 3.7 represents a moderately severe disease activity.

Eighty HIV infected patients were well matched with regard to gender, age, prior influenza vaccinations, actual CD4 count and time on antiretroviral therapy. Over 80% of patients were treated with antiretroviral therapy, the mean time on therapy exceeded five years. The mean CD4 nadir was 166 and 193 cells/mm3 in the intramuscular and intradermal arm respectively. Only 10 patients with a CD4 count of <200 cells / mm3 were included.

Table 1. Baseline characteristics of subjects.

<table>
<thead>
<tr>
<th>characteristics</th>
<th>HCs</th>
<th>anti-TNF</th>
<th>HIV</th>
<th>HSCT</th>
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</tr>
</thead>
<tbody>
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<td>im</td>
<td>id</td>
<td>im</td>
<td>id</td>
<td>im</td>
</tr>
<tr>
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<td>20</td>
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<td>25</td>
<td>25</td>
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<td>36</td>
<td>36</td>
<td>71</td>
</tr>
<tr>
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<td>42 (20-60)</td>
<td>54 (37-71)</td>
<td>53 (36-81)</td>
<td>46 (22-75)</td>
</tr>
<tr>
<td>previously vaccinated,%</td>
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<td>48</td>
<td>28</td>
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<td>61</td>
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<tr>
<td>DAS28, mean</td>
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<td>-</td>
<td>4.0</td>
<td>3.1</td>
<td>-</td>
</tr>
<tr>
<td>CD4, mean, cells/mm3 (range)</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>440 (65-840)</td>
</tr>
<tr>
<td>time since hsct, months (range)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>19 (4-56)</td>
</tr>
</tbody>
</table>

* percentage denotes vaccination status of subjects, not of donors (in case of allograft transplantation) χ²-square test (2-sided) for intramuscular versus intradermal in the four study groups one-way ANOVA for intramuscular versus intradermal in the four study groups HCs: healthy controls; TNF: tumor necrosis factor; HIV: human immunodeficiency virus; HSCT: hematologic stem cell transplantation; DAS28: disease activity score
Twenty-six HSCT patients were well matched in regard to gender, age, prior influenza vaccination and time since transplantation. Underlying diseases were multiple myeloma (n=10), acute myeloid leukaemia (n=7), non-Hodgkin lymphoma (n=3) and other (n=6). The intradermal group had a higher percentage of patients with an allograft (62 vs 23%, p=0.047).

Immune responses
The intradermal vaccination with a low dose resulted in similar postvaccination titers as compared to standard intramuscular vaccination in all four study groups (HC, anti-TNF, HIV and HSCT group). There was a clear hierarchy in postvaccination titers and protection rates for the different study groups (in both intramuscular and intradermal study arms): healthy controls showed the best responses followed by the anti-TNF group, the HIV group and the HSCT group respectively (Figure 1). Multivariate analyses were done in the four separate study groups.

Prevaccination titers were already protective for a considerable percentage of the subjects (figure 2), which can be caused by either natural exposure or previous vaccination. The correlation between previous vaccination and higher prevaccination titers was the strongest for the two antigens that were identical to the ones used in the previous (2004/2005) vaccine: influenza A/H1N1 and B (data not shown). Higher prevaccination titers were associated with higher postvaccination titers in all study groups.

In the HIV group, older age and a CD4-count of less than 200 cells per mm$^3$ were associated with lower postvaccination GMTs when controlling for sex, HIV treatment, previous vaccination and prevaccination titers.

In the HSCT group, no prior vaccination and being within the first year post transplantation were associated with lower postvaccination titers (allotransplantation vs. autotransplantation only showed a trend for lower postvaccination titers). For healthy

Figure 1. Geometric mean titers at day 28 for respectively influenza A/H3N2, A/H1N1 and influenza B. Error bars indicate 95% confidence interval.

HC: healthy control; TNF: tumor necrosis factor; HIV: human immunodeficiency virus; HSCT: hematologic stem cell transplantation; im: intramuscular; id: intradermal

* p < 0.05
controls and the anti-TNF group there were no additional factors associated with lower postvaccination titers.

In concordance with the postvaccination titers, the protection rates were similar after intradermal and intramuscular vaccination in the four study groups, for all three antigens. As depicted in Figure 2, the highest percentages of protective titers were seen in healthy controls (81-100%, depending on vaccination route and antigen). Protection rates were lower in anti-TNF and HIV patients (66-84%) and lowest in HSCT patients (23-62%). All of the healthy controls responded to at least one antigen and 76% to all three antigens contained in the vaccine (Figure 3). In contrast, 42% of all HSCT patients did not respond to any antigen and only 27% responded to all three. Data for the other study groups are depicted in Figure 3.

In a subgroup analysis of patients with the most severe immunodeficiencies, consisting of HIV-infected patients with a CD4 count <200 cells/mm³ (n=10) and HSCT patients...
within the first 11 months following transplantation (n=11), the antibody responses were very poor (postvaccination GMTs of 21, 26 and 13 for respectively A/H3N2, A/H1N1 and influenza B). Of the 21 patients, 6 were vaccinated before and received intradermal vaccination. These six patients yielded remarkable protection rates for patients with a severe immunodeficiency (67-83% (depending on the antigen) vs. 13-27% in the 15 patients not vaccinated before and/or vaccinated intramuscular, p<0.05 for A/H3N2 and influenza B; p=0.09 for A/H1N1).

Safety and adverse reactions

The mean diameter of the wheal caused by the intradermal vaccination was 7.6 mm (SD 1.6 mm), with no significant differences between the study groups. Skin atrophy caused by corticosteroid use in the past and a very dark skin colour made the technique of intradermal vaccine delivery more difficult.

To exclude a recall bias, only study logs that were returned within three months after vaccination were included in the safety analysis. Sixty three percent of the participants (n=125) returned the study log and recorded whether or not they had suffered adverse reactions.

No serious adverse reactions were experienced in the present study. The frequency of adverse reactions after intramuscular vaccination (ranging from muscle pain to fever) reported in this study was high (11-48%). Local reactions, mostly consisting of a transient painless erythema, 48 hours after intradermal vaccination, occurred frequently in the intradermal groups, however significant more often in healthy controls than in immunocompromised patients (p<0.05 Pearson's chi square test, Table 2). The frequency of reported local reactions after intradermal vaccination reflected antibody responses after...
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Table 2. Percentage of subjects that reported side effects following vaccination within each study group. P-values from comparison against healthy controls (Pearson chi-square test).

<table>
<thead>
<tr>
<th>study group</th>
<th>im</th>
<th>p-value</th>
<th>id</th>
<th>p-value</th>
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<tbody>
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<td>18</td>
<td>reference</td>
<td>89</td>
<td>reference</td>
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<tr>
<td>anti-TNF</td>
<td>48</td>
<td>ns</td>
<td>58</td>
<td>ns</td>
</tr>
<tr>
<td>HIV</td>
<td>19</td>
<td>ns</td>
<td>55</td>
<td>ns</td>
</tr>
<tr>
<td>HSCT</td>
<td>11</td>
<td>ns</td>
<td>25</td>
<td>.01</td>
</tr>
<tr>
<td>ICHs (total of three patient groups)</td>
<td>30</td>
<td>ns</td>
<td>52</td>
<td>.038</td>
</tr>
</tbody>
</table>

HCs: healthy controls; TNF: tumor necrosis factor; HIV: human immunodeficiency virus; HSCT: hematologic stem cell transplantation
ICH: immunocompromised hosts

Intradermal influenza vaccination, using only a fifth of the normal dose, leads to similar postvaccination antibody titers and protection rates as compared to standard intramuscular vaccination in immunocompromised patients. Since there is a dose-response relation between the amount of antigen used and the subsequent serologic response, this indicates that intradermal vaccination is more efficient in inducing antibody responses than intramuscular vaccination. [17, 18] This principle was recently proven to be valid in a clinical study. [36] A likely mechanism for this phenomena is that the abundance of antigen presenting cells (APCs) in the dermis reduces the chance that antigens are cleared by circulating pre-existing antibodies or non-specific immunity after intradermal vaccination. In animal studies, intradermal vaccination induced a faster and more profound cellular immune responses in the local lymph node than intramuscular vaccination. [37] Earlier studies that compared reduced dose intradermal influenza vaccination with standard intramuscular vaccination have been criticized for not including a third study arm with a low dose (0.1 mL) delivered intramuscular. [38] Since we anticipated significantly lower protection rates in patients indicated to receive influenza vaccination, such a study arm was not considered to be ethical. [16]
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Patients who, as a result of cellular immunodeficiency, respond with lower postvaccination titers and protection rates to T-cell-dependent vaccines such as the influenza vaccine have the most to gain from a more efficient immune response. [5-7] The underlying T-cell and APC defects, different in nature and severity in the three patient groups of the present study, can be partially overcome by using intradermal vaccine delivery. [39-41] In the present study, only the HIV-group had higher titers after intradermal vaccination. Whether or not this reflects a true different (more efficient) mechanism as opposed to the other study groups is not clear from the present study. The only other study that reported on intradermal influenza vaccination in HIV-infected patients also found a trend for a higher percentage of responders in subjects receiving intradermal vaccination than in those receiving standard intramuscular vaccination. [29]

Several vaccines have been proven to be safe and effective when a reduced dose was delivered intradermally, mostly in immunocompetent subjects. [42-46] Intradermal vaccination in immunocompromised patients has been studied extensively, mostly using the hepatitis B virus (HBV) vaccine. A meta-analysis of intradermal HBV vaccination in patients with chronic kidney disease showed higher protection rates after intradermal administration of the vaccine than after standard intramuscular vaccinations. This difference was no longer detected during the follow up (6-60 months after completion of the vaccination schedule). [42]

Although in theory intradermal delivery of vaccines could save up to 240 million of the 300 million influenza vaccine doses distributed annually worldwide, this is not likely to be a realistic scenario. [31] The more demanding administration technique will hamper mass vaccination campaigns and the frequency and severity of adverse effects following intradermal vaccination in immunocompetent subjects are such that this technique is unsuitable for routine influenza vaccination. Immunocompromised patients, however, form a relatively small subgroup of those indicated for annual influenza vaccination and adverse reactions are significantly milder and less frequent in these patients than in healthy subjects. The overall frequency of adverse reactions might be overestimated in the present study, since a proportion of the subjects who did not return their study log, reported that they did so because they had no adverse events to report.

An interesting finding in the present study was that the presence of a local skin reaction correlated with the magnitude of the antibody response to at least one out of the three vaccine antigens. The absence of a local skin reaction within the first 48 hours following vaccination identified patients that did not develop an adequate response (measured 4 weeks later). In our study this subgroup was too small to evaluate the effect of booster vaccination. The skin reaction was interpreted as a delayed type hypersensitivity reaction, which could be directed at either of the three hemagglutinin or neuraminidase antigens or even the traces of chicken egg or preservatives contained in the vaccine.
Of further interest were the remarkably high postvaccination protection rates after intradermal vaccination in a subset of severely immunocompromised patients who were vaccinated before. A greater effect of intradermal vaccination in a primed population as compared to a ‘virgin’ population was already reported 50 years ago. [20] Again the subset of (combined HIV and HSCT) patients was too small in the present study to draw any firm conclusions. Still, the fact that up to 83% of the primed patients who received intradermal vaccination, had protective postvaccination titers suggests that intradermal vaccination could be the most optimal route of (annual) vaccination in patients with a severely impaired cellular immunity.

In conclusion, dose sparing intradermal influenza vaccination is a feasible alternative for the routine practice of intramuscular vaccination in several groups of immunocompromised subjects, including patients treated with anti-TNF, HIV-infected patients and HSCT patients. In these patients the local skin reaction upon vaccination may be used as a predictor for the outcome of vaccination, identifying the patients who most likely would benefit from a booster vaccination. Intradermal vaccine delivery should be further explored, especially for annual influenza vaccination of severely immunocompromised patients.

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