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ORIGINAL ARTICLE

The antibody response to pandemic H1N1 2009 influenza vaccine in adult organ transplant patients

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Summary

Limited data are available regarding antibody response and the safety of the monovalent influenza A H1N1/09 vaccine for immunocompromised patients. In this study, the humoral response to this vaccine in solid organ transplant (SOT) recipients and healthy individuals was evaluated. Eighty-two SOT recipients and 28 healthy individuals received two doses of the influenza A H1N1/09 AS03 adjuvanted vaccine containing 3.75 mg of haemagglutinin at a 3- to 4-week interval. Serum samples were drawn at baseline and 3–4 weeks after the first and second vaccine doses. Seroprotective titres were measured with a haemagglutination inhibition. After the first dose seroprotective titres were observed in 69% of the SOT patients and in 96% of the healthy controls ($P = 0.006$), and increased after the second dose to 80% and 100%, respectively ($P = 0.003$). All controls and 77% of the SOT recipients achieved a ≥4-fold titre rise after the first immunisation ($P = 0.005$). The vaccine was well tolerated and no acute rejection was observed. Influenza A H1N1/09 vaccine elicited a protective antibody response in the majority of SOT recipients, but the response was lower when compared with controls. A second dose significantly improved vaccine immunogenicity in SOT recipients.
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Introduction

Influenza virus infection in transplant recipients is associated with a higher rate of complications such as viral pneumonia, secondary bacterial pneumonia and on rare occasions, acute allograft rejection [1–3].

During the summer of 2009, it became apparent that the novel influenza A H1N1 virus had pandemic potential [4,5]. The 2009 H1N1 virus has a unique re-assortment of swine, avian and human influenza genes of both North American and Eurasian origin that has not been identified previously in either swine or human populations [4,6]. Pandemic H1N1 influenza has been described as being most common among young people. It is a particularly severe disease during pregnancy and among more traditional high-risk groups for influenza infection, including transplant recipients. In a recent multicentre study on organ transplant patients diagnosed with 2009 pandemic H1N1 influenza 40% of adult recipients developed pneumonia, 17.5% were admitted to ICUs and 7% died [7].

During the autumn of 2009, the Swedish National Board of Health and Welfare published influenza H1N1 vaccination recommendations. It was advised patients with risk factors, including immunosuppression, were to be administered two doses of an inactivated monovalent vaccine, Pandemrix® (GlaxoSmithKline, Brentford, United Kingdom), with a minimum of 3 weeks between the doses. Among healthy subjects, the monovalent influenza A H1N/09 vaccine has been shown to have a high immunogenicity [8]. In contrast, little data are available regarding the immunologic response to this vaccine in organ transplant recipients [9,10]. Vaccination against annual seasonal influenza, which has been the standard of care in most transplant centres, has in different studies shown various

antibody responses. Immunogenicity of annual influenza vaccine has been reported as efficacious in several studies [11,12], while others have found a suboptimal response to influenza vaccine in organ transplant patients [13–15].

The risk of rejection and other adverse effects with adjuvanted influenza A H1N1/09 vaccine in transplant settings is largely unknown. However, previous studies in healthy individuals showed that the frequency of adverse events was significantly higher after vaccination with adjuvanted influenza A H5N1 vaccine when compared with nonadjuvanted vaccines [16,17]. Recently, the development of de novo anti-HLA antibody after pandemic H1N1 and seasonal influenza vaccination in kidney transplant recipients have been reported [18].

The aim of this study was to analyze the antibody response to the monovalent influenza A H1N1/09 vaccine in organ transplant patients when compared with healthy controls. In addition, signs of acute rejection and adverse events were registered.

Patients and methods

Patients, controls and procedures

A total of 82 consecutive solid organ transplant (SOT) patients from the outpatient clinic at the Transplant Institute, Sahlgrenska University Hospital, were included in the study. The healthy controls were 28 staff members from the Transplant Institute. Patients and the controls were vaccinated between October and November 2009 according to the clinical guidelines with two doses of monovalent influenza A H1N1/09 vaccine, Pandemrix®. The second dose was administered 3–4 weeks after the first. The study's inclusion criteria was vaccination with Pandemrix®, a serum sample drawn at baseline and at least one additional sample 3–4 weeks after the first or second vaccine dose.

The demographic data are shown in Table 1. The majority of the patients were kidney transplanted. Newly transplanted patients, within in a month, were not vaccinated. Among the included patients, only five were vaccinated within 3–9 months after transplantation. There was a great diversity with respect to basal immunosuppression among the patients. The renal transplant patients were most often on triple therapy (31 of 49 patients, 63%) consisting mainly of calcineurin inhibitors (CNI), mycophenolat acid (MPA) and steroids. In contrast, the majority of the liver and heart transplanted patients were treated with only two drugs (63% vs. 86%). The liver transplant patients were treated mainly with CNI and steroids, while the heart transplant patients had a nonsteroid regimen of CNI + azathioprine or MPA or CNI + proliferation signal inhibitors. Three patients received anti-rejection treatment with steroids 2–5 months before vaccination. The immunosuppression remained unchanged during the vaccination period except for one patient. Medical records were reviewed 1 year after completion of vaccination in search for graft dysfunction, performed biopsies and anti-rejection treatment. Thoracic transplanted patients were monitored by control biopsies during the first 3 (lung recipients) to 12 months (heart recipients), and thereafter only on clinical indication. Liver and kidney transplanted patients were only biopsied on clinical indication.

Table 1. Demographics of the study population.

Characteristics	Transplant recipients (n = 82)	Healthy controls (n = 28)
Age years, median (range)	60 (28–80)	43 (21–68)
Gender F/M	35/47	21/13
Organ transplanted		
Kidney	49	
Liver	17	
Heart	7	
Lung	2	
Kidney/heart, lung/liver	3/2/1	
Keratolimbal stem cell	1	
Years since transplantation Median (range)	6 (0.25–24)	

At the time of the first vaccination subjects received a questionnaire for reporting of side effects. The questionnaire was to be returned after completion of the vaccinations. The study was approved by the Regional Ethical Review Board in Gothenburg (number 590–10) and all patients gave their written informed consent to participate.

Vaccine

The Influenza A H1N1/09 vaccine used in Sweden was Pandemrix®, an inactivated split influenza virus vaccine, containing antigen equivalent to the A/California/07/2009 (H1N1) derived strain (NYMC X-179A): 3.75 μg and AS03 adjuvant composed of squalene (10.69 mg), DL-α-tocopherol (11.86 mg) and polysorbate 80 (4.86 mg). The vaccine was administered by an intramuscular injection into the deltoid muscle. Eight patients out of 82 and one of the controls also received one dose of the seasonal influenza vaccine 2009 Fluarix®. This vaccine was given concomitantly with the second dose of Pandemrix®.

Haemagglutination inhibition assay

Serum samples were drawn at baseline and 3–4 weeks after the first and second immunisation. Pre and postvaccination samples were analyzed simultaneously by haemagglutination (HI) inhibition assay. To abolish

nonspecific inhibitors in serum samples, sera were treated overnight with a receptor destroying enzyme (RDE; Denka Seiken, Tokyo, Japan) according to the instructions of the manufacturer. Subsequently, the mixture was incubated at 37 °C for 30 min to inactivate RDE and complement. A phosphate buffered saline (PBS) solution was then added to achieve a 1:10 dilution, followed by a serial twofold titration (final dilution 1:1,280). Dilutions of sera (50 μl) were then incubated with 25 μl PBS containing four haemagglutination (HA) units of pandemic influenza virus (A/California/7/2009 NYMC X-179A H1N1) for 15 min at room temperature. Subsequently, 50 μl of a 0.5% hen red blood cell suspension was added to the mixture and incubated for 30–45 min before evaluation of HA. The HI titre was judged as the reciprocal of the last dilution that inhibited HA. Titres of 40 and above were considered to be a positive antibody response.

Statistical analysis

Frequency of vaccinated patients and controls with protective antibody titres (≥1:40) and with a ≥4-fold titre rise after vaccination and the frequency of adverse events were compared using Fisher's exact test. A *t*-test was used to compare the mean antibody titres between the two groups. Analysis of co-variance (ANCOVA) adjusted for the effects of age and gender was used to correct for these group differences. McNemar's test was used for a comparison of the proportion of responders after the first and second vaccine dose.

Results

All patients and controls received two doses of vaccine. In the majority of patients (68 of 82) and controls (20 of 28), the serum samples were analyzed both after the first and second vaccine dose. In 13 of 82 patients and 8 of 28 controls the serum samples were analyzed only after the first vaccine dose and in 6 of 82 patients only after the second vaccine dose.

The efficacy of influenza vaccination was studied on the basis of the following three indices: percentages of individuals who reached protective titres (≥1:40), percentages of individuals with a ≥4-fold titre rise and the mean titres reached in patients and controls.

Before immunisation, three patients (born 1946, 1951 and 1973) and two controls (born 1959 and 1989) had protective antibody titres against the Influenza A H1N1/09 virus.

After the first vaccine dose, 69% of the patients and 96% of the controls responded with protective titres ($P = 0.006$). After the second immunisation, the frequency of responders increased to 80% in the patient group and 100% in the control group ($P = 0.003$) (Fig. 1). In SOT recipients a significant increase in the proportion of responders with protective titres (≥1:40) was detected after the second vaccine dose when compared to after the first dose ($P = 0.001$).

The percentages of patients and controls with a ≥4-fold titre rise are shown in Fig. 2. All controls achieved a ≥4-fold titre rise after the first immunisation in contrast to 77% of the SOT patients ($P = 0.005$). After the second immunisation, the percentage increased to 81% of the SOT patients.

The distribution of the magnitude of the H1N1 antibody titre rise is shown in Fig. 3. The control individuals responded with significantly higher titres after both the first ($P < 0.001$) and second dose ($P < 0.001$) when compared with patients (Fig. 3). The patients were older than

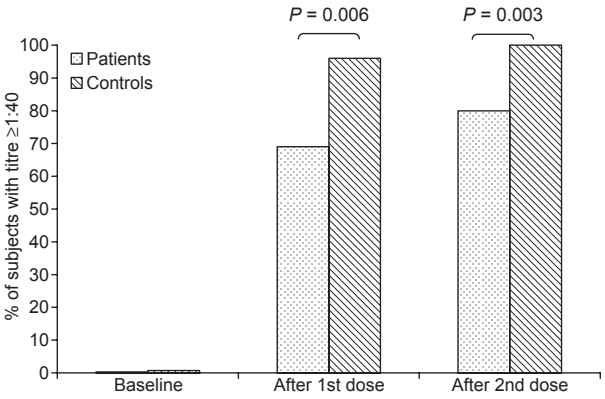

Figure 1 Percentages of organ transplant patients versus healthy controls with protective serum haemagglutination inhibition antibody titres (≥1:40) at baseline and 3–4 weeks after both first and second dose of adjuvanted influenza A H1N1/09 vaccine.

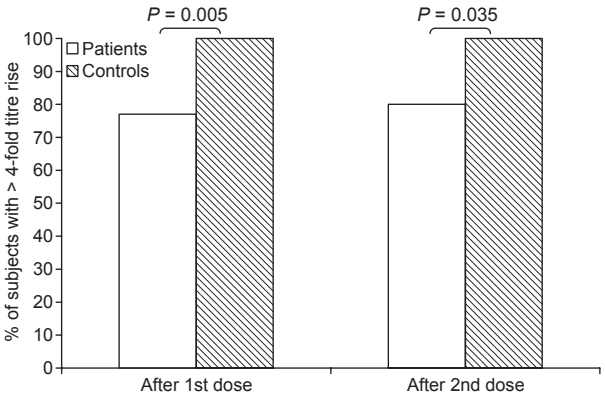

Figure 2 Percentages of organ transplant patients versus healthy controls with ≥4-fold titre rise, measured by serum haemagglutination inhibition antibody titres and 3–4 weeks after both first and second dose of adjuvanted influenza A H1N1/09 vaccine.

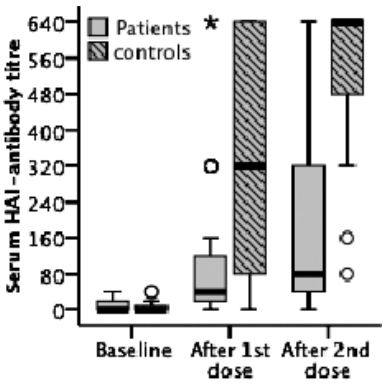

Figure 3 The box plot showing the distribution of the magnitude of H1N1 antibody titre rise in organ transplant patients versus healthy controls at baseline and 3–4 weeks after both first and second dose of adjuvanted influenza A H1N1/09 vaccine. The box plot represents the 25th–75th percentile, the dark line is the median and the extended bars represent the 10th–90th percentile. Asterisk and ring represents suspected outliers.

the controls and male gender dominated (Table 1). Therefore, ANCOVA, controlling the effect for age and gender, was performed and the significance remained.

Thirteen of 82 patients did not develop protective titres; i.e. nonresponders. This group of patients were older than responders (mean 61 vs. 54 years of age). Of these 13 nonresponders, 12 (92%) were treated with triple immunosuppressive therapy compared with 24 of 69 (35%) of the responders.

The glomerular filtration rate (GFR) was measured within 1 year of the study start in 78 of 82 patient participants. The measurement used was either CrEDTA or Iohexol. The omitted four patients all had normal s-creatinine levels. Fifteen patients had stadium 4 chronic kidney disease (GFR 15–29 ml/min/1.73 m^2), and four patients were at stadium 5 (GFR <15 ml/min/1.73 m^2). We found a significant difference in patients with GFR ≥30 ml/min, where 87% responded with protective antibody titres after the second vaccine dose, compared with 61% of patients with GFR <30 ml/min ($P = 0.036$). The same tendency was seen when analyzing the fourfold titre rise. Only 67% of patients with GFR < 30 ml/min responded with a ≥4-fold titre rise compared with 85% of patients with GFR ≥ 30 ml/min ($P = 0.16$).

No patient developed acute rejection during 1 year of follow-up after vaccination. In total only three patients (one liver, one kidney, one heart) underwent biopsy and none of the biopsies revealed acute rejection. The kidney transplanted patient had a stable renal function 2 months after vaccination, but reduced renal function at a check-up 5 months after vaccination and a biopsy showed transplant glomerulopathy interpreted as chronic rejection.

Table 2. Frequency of side effects after the first and second adjuvanted influenza A H1N1/09 vaccine dose reported in questionnaires by transplant patients and healthy controls.

	Patients *n* = 75		Controls *n* = 27	
Reported symptoms	First dose	Second dose	First dose	Second dose
Local symptoms* *n* (%)	51 (68)	41 (55)	16 (59)	12 (44)
Myalgia *n* (%)	16 (21)	15 (20)	13 (48)	12 (44)
Fever *n* (%)	10 (13)	13 (17)	8 (30)	6 (22)
Cough *n* (%)	4 (5)	3 (4)	1 (4)	1 (4)
Headache *n* (%)	2 (3)	4 (5)	4 (15)	4 (15)
Unusual symptoms†	2 (3)			

*Tenderness, redness and pain at the place of injection.
†Sudden deafness (*n* = 1), vertigo, herpes simplex and nose bleeding (*n* = 1).

A switch from cyclosporine to tacrolimus was performed but the renal function continued to decline and the patient started dialysis 14 months after vaccination.

Side effects were assessed using the questionnaire distributed to all participants. A total of 76 of 82 (93%) of the patients and 27 of 28 (96%) of the controls returned their questionnaires. The frequency of side-effects after the first and second vaccine dose are shown in Table 2. Myalgia was significantly more frequently reported by controls, both after the first ($P = 0.012$) and the second vaccine dose ($P = 0.021$), when compared with patients.

One serious event was recorded. A 73-year-old man with previously impaired hearing developed unilateral sudden deafness 1 week after the first vaccine dose. Examination revealed a sensorineural hearing loss.

Discussion

The majority of SOT patients in our study, vaccinated with monovalent influenza A H1N1/09 vaccine, Pandemrix®, developed a protective antibody response. However, the response was lower when compared with controls. A second dose of the vaccine significantly improved the antibody response among the transplant recipients.

Little has been published on the efficacy of the Influenza A H1N1/09 vaccine in immunocompromised patients. Until May 2011, few papers have been published describing antibody responses in adults SOT patients [9,10]. Manuel *et al.* observed seroconversion rate in 15 (52%) out of 29 kidney and liver transplant recipients both after the first and second dose of monovalent influenza A H1N1/09 vaccine. A single dose of the same vaccine in heart transplant recipients was used in an uncontrolled study and the seroprotection rate reached

32% (15 of 47) [10]. The substantially lower vaccine responses in these studies compared with our study might depend on factors such as the time from transplantation, various immunosuppression treatment regimens and organ transplanted. SOT patients in our study also seem to respond better than patients with B-cell malignancies and allogen SCT who participated in the two other studies [19,20].

Our results support the recommendation by the Swedish National Board of Health and Welfare to use two doses of monovalent influenza A H1N1/09 vaccine in organ transplant patients. In patients with haematological malignancies, de Lavallade *et al.* supported the recommendation to use two doses of monovalent influenza H1N1/09 vaccine as only 39% of patients with B-cells malignancy and 46% with allogen SCT reached protective antibody levels after the first dose of vaccine. The second vaccine dose enhanced the responses and 68% of patients with B-cells malignancies ($n = 39$) and 73% of patients with allogen SCT ($n = 26$) reached protective antibody levels.

Only few vaccine recipients in our study received a seasonal influenza vaccination at the same point in time as their second dose of Pandemrix®. Therefore, the enhanced immune response after the second vaccine dose in our study was probably not related to boosting with a seasonal influenza vaccine.

Previous studies of SOT patients reported higher post-vaccination seroprotective rates after a single dose of annual influenza vaccine when compared with our study [12]. However, an exact comparison is difficult as the frequency of individuals with pre-existing antibodies against Influenza A H1N1/09 is low in the population, except for individuals 80 years or older [21]. In our study only 4.5% of the participants had H1N1-antibodies at baseline which might be cross-reactive antibodies, although an asymptomatic or subclinical influenza caused by Influenza H1N1/09 cannot be excluded. The finding that very few individuals had pre-existing antibodies is consistent with the large genetic difference between the novel influenza A (H1N1) virus and recent seasonal human H1N1 viruses [22].

We found a decreased response in our patients with severe renal impairment. In contrast, Dikow *et al.* found a good immune response to H1N1 adjuvant vaccine in a haemodialysis population [23]. The tendency of lower antibody response among our patients with severe renal impairment may not only reflect an immune dysfunction secondary to chronic renal failure, but in addition this lower response may be related to the immunosuppressive treatment. Interestingly, among the 13 nonresponders all but one was treated with triple immunosuppressive therapy.

A weakness of our study is the higher median age in the cohort of patients compared with the controls. However, when adjusting for the effect of age, the significance between the groups concerning the titre rise after the first and second vaccine dose remained.

It has been hypothesised that immunization with influenza pandemic vaccine may induce an immune response triggering rejection episodes. The Pandemrix® vaccine, used in this study, contains a squalene-based adjuvant (AS03). Little information is available regarding the immunomodulatory activity of squalene. Anti-HLA-antibodies have been detected after Pandemrix® immunization in solid organ transplanted patients indicating a possible immunomodulatory effect of the vaccine [18]. Recently Vistoli *et al.* reported a case of acute rejection in a pancreas transplanted patient after pandemic influenza vaccination [3]. This patient developed antibodies against several HLA class I and II antigens (including donor-specific antibodies) shortly after influenza A H1N1 vaccination.

Anti-HLA-antibodies were not measured in our study; however, in our 82 recipients no episode of acute rejection was recorded after vaccination during the 1 year observation period. One of our renal recipients developed chronic rejection after vaccination. Whether this chronic rejection process was attenuated or not by the influenza vaccination remains unclear.

Only one serious event was registered after influenza A H1N1/09 vaccination, namely sudden deafness. Sensorineural hearing loss is a rare complication after vaccination and has been described after H1N1 influenza vaccine in a single case [24]. The higher frequency of headache and myalgia noted in healthy individuals compared with SOT patients may have been elicited by a more pronounced immune system response after vaccination in immunocompetent individuals. The safety profile of the vaccine was acceptable and in agreement with other studies on healthy individuals and immunocompromised patients [8,19].

In summary, vaccination with the influenza A H1N1/09 vaccine was well tolerated, except for one case of sudden deafness, and was effective in SOT patients. Furthermore, our results support the recommendation for two doses of monovalent H1N1 influenza for immunocompromised patients to induce a protective immune response against 2009 H1N1 influenza.

Authorship

MF, VF and BS: designed and performed the study. MF: collected the data. BS: performed virological analyses. MF, MS, BS and VF: analyzed the data and wrote the paper.

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